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<p>(54) Title: METHOD FOR DIAGNOSING AND TREATING CANCER</p> <p>(57) Abstract</p> <p>The present invention describes conjugates of growth factors and alpha-emitting radionuclides which are suitable for detecting and treating cancer. Also provided are methods for treating cancer utilizing conjugates of growth factors and non-radioactive iodine, conjugates of growth factors and an oxyanion of a metal, and conjugates of a growth factor and a radioactive isotope.</p>			

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Description

METHOD FOR DIAGNOSING AND TREATING CANCER

5 Statement of Government Interest

This invention was made with government support under contract DE-A606-76RLO 1830, awarded by the U.S. Department of Energy. The government has certain rights in the invention.

10 Technical Field

The present invention relates generally to methods for diagnosing and treating cancer.

Background of the Invention

15 Cancer accounts for one-fifth of the total mortality in the United States, and is the second leading cause of death after cardiovascular diseases and stroke. The three leading types of tumors found in man are lung, prostate, and colorectal cancer, and the three leading types of tumors found in women are breast, lung, and colorectal cancer. Common therapeutic approaches for the
20 treatment of cancer generally involve the surgical removal of solid tumors, followed by chemotherapy and/or radiotherapy. One disadvantage of this general approach, however, is that most chemotherapeutic or radiotherapeutic agents are not tumor-cell specific, thus damaging normal tissue during the course of treatment.

25 Various methods have been utilized in order to more effectively direct or target therapeutic agents to tumor cells. For example, many tumor cells have an increased number of certain cell surface antigens as compared to normal cells. This difference between tumor and normal cells may be exploited in order to more effectively target therapeutic agents to tumor cells. More specifically,
30 targeting agents such as monoclonal antibodies may be used to specifically target and bind to the tumor cells, resulting in the localization and internalization of the therapeutic agents. For example, monoclonal antibodies such as the anti-gp160 antibody for human lung cancer (see Sugiyama et al., "Selective Growth Inhibition of Human Lung Cancer Cell Lines Bearing a Surface Glycoprotein gp160 by ¹²⁵I-Labeled Anti-gp160 Monoclonal Antibody," *Cancer Res.* 48:2768-2773, 1988), a "TNT-1" monoclonal antibody for human cervical carcinoma (see Chen et al.,
35 "Tumor Necrosis Treatment of ME-180 Human Cervical Carcinoma Model with

"¹³¹I-Labeled TNT-1 Monoclonal Antibody," Department of Pathology, University of Southern California School of Medicine, Los Angeles, California), and antibodies against the epidermal growth factor receptor for KB carcinoma (see Aboud-Pirak et al., "Efficacy of Antibodies to Epidermal Growth Factor Receptor Against KB Carcinoma *In Vitro* and in Nude Mice," *J. National Cancer Institute* 80(20):1605-1611, 1988) have been used to specifically localize tumor cells. Monoclonal antibodies, however, are disadvantageous because they are typically developed in mouse systems, and injection of such antibodies into humans results in the generation of an extensive immune response against the antibody itself, thus limiting its effectiveness in killing tumor cells.

In order to kill tumor cells, targeting agents have been coupled to various chemotherapeutic agents including, among others, ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* toxin, *Shigella* toxin, and Pokeweed antiviral toxin (see U.S. Patent No. 4,545,985; see also Jansen et al., "Immunotoxins: Hybrid Molecules Combining High Specificity and Potent Cytotoxicity," *Immunological Review* 62:185-216, 1982; see also Thorpe and Ross, "The Preparation and Cytotoxic Properties of Antibody-Toxin Conjugates," *Immunological Review* 62:119-158). Similarly, various radiotherapeutic agents have also been utilized to kill tumor cells including, for example, the beta emitters ¹³¹I, ⁶⁷Cu, ¹⁸⁶Re, and ⁹⁰Y. Beta emitters, however, are disadvantageous because of their low specific activity, low linear energy transfer, low dose rates (allowing for cell repair of radiation damage), damage to surrounding normal tissues, and in some cases the lack of an associated imageable photon (e.g., yttrium-90).

The present invention overcomes the disadvantages discussed above, and further provides other related advantages.

Summary of the Invention

The present invention provides reagents and methods for detecting and treating cancer. Within one aspect of the present invention, a conjugate of a growth factor and an alpha-emitting radionuclide is provided, the growth factor being capable of specifically binding to a defined population of cancer cells. Within various embodiments, the growth factor is coupled to the alpha-emitting radionuclide by a linker, such as a short polycarbon compound, to separate the alpha-emitting radionuclide from the growth factor. Preferred linkers may be selected from the group consisting of disulfides, dicarboxylic acids, and multi-carbon chain linkers (polycarbons). A particularly preferred linker is hexamethylene diamine. This linker may be coupled to a portion of the growth

- factor selected from the group consisting of the N-terminus and the C-terminus. In addition, within other embodiments of the invention, the alpha-emitting radionuclide is bound to a sequestering agent, such as, for example, a macrocyclic complexing agent. Preferred macrocyclic complexing agents include crown ethers 5 such as a 21-crown-7 or an 18-crown-6 ether. Within another aspect of the present invention, a pharmaceutical composition is provided comprising a conjugate of a growth factor and an alpha-emitting radionuclide, and a pharmaceutically acceptable carrier or diluent, the growth factor being capable of specifically binding to a defined population of cancer cells.
- 10 Within various embodiments of the present invention, the alpha-emitting radionuclide is selected from the group consisting of lead-212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, polonium-210, astatine-211, radium-223, radium-224, and actinium-225.
- 15 Within another aspect of the present invention, a conjugate of a growth factor and non-radioactive iodine is provided, the growth factor being capable of specifically binding to a defined population of cancer cells. Pharmaceutical compositions are also provided, comprising a conjugate of a growth factor and non-radioactive iodine, and a pharmaceutically acceptable carrier or diluent, the growth factor being capable of specifically binding to a 20 defined population of cancer cells.
- 25 Within other aspects of the invention, a method for treating cancer in warm-blooded animals is provided, comprising administering to a warm-blooded animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, a conjugate of a growth factor and non-radioactive iodine, a conjugate of a growth factor and yttrium-90, or a conjugate of a growth factor and an oxyanion of a metal, the growth factor being capable of specifically binding to a defined population of cancer cells. Within particularly preferred embodiments of the invention, the above-described method further comprises, prior to the step of administering an effective amount of a conjugate, 30 administering an unlabeled growth factor capable of specifically binding to the defined population of cancer cells, in an amount sufficient to mask growth factor receptors in healthy tissues of the animal.

35 Within yet another aspect of the present invention, a method for detecting cancer is provided, comprising the steps of (a) administering to a warm-blooded animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, the growth factor being capable of specifically binding to a defined population of cancer cells; and (b) detecting the presence and

location of the conjugate within the warm-blooded animal and therefrom determining the presence of cancer.

Within another aspect of the present invention, a method for detecting the presence of cancer in warm-blooded animals is provided, comprising
5 the steps of (a) administering to the animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of a conjugate of the growth factor and a radioactive isotope which emits gamma radiation, and (c) detecting
10 the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of cancer. Within one embodiment, the radioactive isotope is selected from the group consisting of rhenium-186, technetium-99m, iodine-131, selenium-75, iodine-123, iodine-125, iodine-124, indium-111, copper-67, radium-223, gold-198, yttrium-90, chromium-51, iron-52,
15 copper-64, gallium-67, gallium-66, gallium-72, gallium-68, zirconium-89, ruthenium-97, lead-203, rhodium-105, rhenium-188, gold-199, astatine-211, bromine-76, bromine-77, fluorine-18, bismuth-206, mercury-197, and mercury-203.

Within yet another aspect of the present invention, a method for diagnosing and treating cancer in warm-blooded animals is provided, comprising
20 the steps of (a) administering to the animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of conjugate of the growth factor and a radioactive isotope which emits gamma radiation, (c) detecting the presence
25 and location of the conjugate within the warm-blooded animal and therefrom determining the presence of the cancer, and (d) administering an effective amount of a second conjugate of a growth factor and a radioactive isotope or non-radioactive iodine, such that the cancer is treated.

Within another aspect of the present invention, a method for
30 diagnosing and treating cancer in warm-blooded animals is provided, comprising the steps of (a) administering to the animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of a first conjugate of a growth
35 factor and a radioactive isotope which emits gamma radiation, (c) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of the cancer, and (d) administering an

- effective amount of a second conjugate of a growth factor and a cytotoxic metal ion, such that the cancer is treated. Within one embodiment, the cytotoxic agent is an oxyanion of a metal selected from the group consisting of manganese, technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium.
- 5 Within another embodiment, the cytotoxic agent is an alpha particle emitting radioactive isotope selected from the group consisting of lead-212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, radium-224, and actinium-225.

Within yet other embodiments of the above invention, the growth factor is selected from the group consisting of epidermal growth factor, transforming growth factor - alpha, fibroblast growth factors, insulin-like growth factor I and II, and nerve growth factor.

These and other aspects of the present invention will become evident upon reference to the following drawings and detailed description.

15

Brief Description of the Drawings

FIGURE 1 is a table listing various radioactive nuclides which emit alpha-particle radiation.

FIGURE 2 schematically illustrates a decay series starting with 20 Cm-243. Radium-223 is a member of this decay series.

FIGURE 3 is a graph which illustrates the iodination profile of ^{131}I to epidermal growth factor.

FIGURE 4 is a graph which compares cells treated with ^{131}I , ^{131}I -epidermal growth factor, and epidermal growth factor alone.

25 FIGURE 5 is a graph which illustrates the effects of various concentrations of ^{131}I -epidermal growth factor on A431 cells.

FIGURE 6 is a graph which illustrates the effects of various concentrations of ^{131}I -epidermal growth factor on L cells.

30 FIGURE 7 is a graph which illustrates the effects of various concentrations of non-radioactive iodine-epidermal growth factor on A431 cells.

FIGURE 8 is a graph which illustrates the effects of various concentrations of non-radioactive iodine-epidermal growth factor on L cells.

Detailed Description of the Invention

35 As noted above, the present invention provides reagents for detecting and treating cancer. These reagents generally comprise a conjugate of a growth factor and an alpha-emitting radionuclide, a conjugate of a growth factor

and non-radioactive iodine, or any of a number of growth factor conjugates which are described in more detail below, the growth factor being chosen such that it is capable of specifically binding to a defined population of cancer cells.

Many growth factors known to one of ordinary skill in the art may 5 be utilized within the present invention. Representative examples include platelet derived growth factors, transforming growth factor-beta, interleukins (*i.e.*, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, or IL-9), granulocyte-macrophage colony stimulating factor (GMCSF), erythropoietin, tumor necrosis factor, endothelial cell growth factor, platelet basic proteins, capillary endothelial cell 10 growth factor, cartilage-derived growth factor, chondrosarcoma-derived growth factor, retina-derived growth factor, hepatoma derived growth factor, bombesin, and parathyroid hormone. Particularly preferred growth factors include epidermal growth factor, transforming growth factor - alpha, fibroblast growth factors, insulin like growth factor I and II, and nerve growth factor.

15 The growth factor should be selected such that it is capable of specifically binding to a defined population of cancer cells which include, for example, preneoplastic cells, premetastatic cells, and tumor cells (both benign and malignant). As will be understood by one of ordinary skill in the art, a defined population of cancer cells may generally be differentiated from normal cells based 20 upon the greater number of growth factor receptors on the cell surface. Consequently, within the context of the present invention a growth factor may be defined to be "specifically binding" to a defined population of cancer cells if this population of cells has greater than approximately two times the number of growth factor receptors on its surface as compared to normal cells, and preferably 25 greater than five to ten times the number of growth factor receptors. In addition, this difference in the number of growth factor receptors on cancer cells, as compared to normal cells, may be exploited in order to more specifically target growth factor conjugates. In particular, the number of growth factor receptors on cells in healthy tissue may be determined, and compared to the number of growth 30 factor receptors on cancer cells. As described in more detail below, unlabeled growth factor capable of specifically binding to a defined population of cancer cells may then be administered in an amount sufficient to mask growth factor receptor sites on the normal cells of healthy tissues, in order to mask the less abundant growth factor receptors on normal cells (if present) prior to the addition 35 of a conjugated growth factor.

The number of growth factor receptors on a cell may be readily determined based upon the ability of the cell to bind to the growth factor

receptor's substrate. For example, assays such as radioreceptor binding assays which determine the quantity of receptor substrate that binds to a cell over the course of time may be readily utilized to determine the number and type of cell surface receptors (*see, for example*, Ladda et al., *Anal. Biochem.* 93:286-294, 1979).

- 5 Briefly, utilizing radiolabeled growth factor and membrane preparations isolated from both normal and tumor cells, one can readily determine both growth factor receptor number and affinity by a standard competitive binding assay followed by a Scatchard plot analysis (*see* Scatchard, *Anal. N.Y. Acad. Sci.* 51:660-672, 1949).

In order to determine which growth factor conjugate would be the
10 most effective therapeutically or diagnostically, within one embodiment the cells of interest (e.g., tumor cells) are removed from the patient. The removal of cells may typically be accomplished through surgical procedures, although many other methods may also be utilized, dependent of course on the type of tumor and its location. Once the tumor cells have been removed, they may be maintained in an
15 *in vitro* culture using conventional media (*see, for example*, "Media Formulations," ATCC Cell Lines & Hybridomas, 1988). The number and type of receptors may then be readily determined using methods described above; and a growth factor conjugate selected on the basis of its ability to specifically bind to the tumor cells. Additionally, the therapeutic (or diagnostic) effectiveness of the growth factor
20 conjugate upon tumor cells may be readily determined by *in vitro* assays. A representative assay is described below in Examples 1 and 2.

Alternatively, within another embodiment growth factor conjugates may be utilized for therapeutic or diagnostic purposes based only upon the known characteristics of certain tumors. For example, certain types of tumors such as
25 human epidermal carcinomas are already well defined, and have been shown to possess abnormally high numbers of epidermal growth factor receptors (*see* Berger et al., "Epidermal Growth Factor Receptors in Lung Tumors," *J. Pathology* 152:297-307, 1987; Dotzlaw et al., "Epidermal Growth Factor Gene Expression in Human Breast Biopsy Samples: Relationship to Estrogen and Progesterone
30 Receptor Gene Expression," *Cancer Res.* 50:4204-4208, 1990; Maddy et al., "Epidermal Growth Factor Receptors in Human Prostate Cancer: Correlation with Histological Differentiation of the Tumor," *Br. J. Cancer* 60:41-44, 1989; Liberman et al., "Expression of Epidermal Growth Factor Receptors in Human Brain Tumors," *Cancer Res.* 44:573-760, 1984; Neal et al., "Epidermal Growth
35 Factor Receptors in Human Bladder Cancer: Comparisons of Invasive and Superficial Tumors," *Lancet* 1:366-368, 1985; and Moorgren et al., "Epidermal Growth Factor Receptors in Colorectal Carcinoma," *Anticancer Res.* 10:605-612,

1990). Thus, an epidermal growth factor conjugate may be readily applied to an epidermal carcinoma without the need to first determine which growth factor to use.

Similarly, Interleukin-2 receptors are expressed by abnormal T cells
5 in patients with certain lymphoid malignancies or autoimmune disorders, but not by resting cells. For example, HTLV-I associated adult T-cell leukemia cells constitutively produce large numbers of IL-2 Tac receptors (see Waldmann, *Cancer Surveys* 8(4):891-903, 1989, see also Waldmann, *J. Natl. Canc. Inst.* 81(12):914-923, 1989). Once a malignancy has been classified as an HTLV-I
10 associated adult T-cell leukemia, an IL-2 growth factor conjugate may be utilized therapeutically without the need to further classify the malignancy as discussed above.

Similarly, a combination of growth factor conjugates may be utilized based upon the known distribution of tumor types in a given disease. For
15 example, if 80% of human lung tumors express growth factor receptor type A, 15% of human lung tumors express growth factor receptor type B, and the remaining 5% of human lung tumors express growth factor type C; a conjugate may be prepared for the treatment of lung cancer comprising a combination of growth factors conjugates A, B, and C.

Within one aspect of the present invention the growth factor is conjugated to an alpha-emitting radionuclide. Alpha-emitting radionuclides are particularly preferred because they have short range (35-70 μm through solid tissue and 35-700 μm through lung tissue), and are extremely efficient in killing cells. On the average, only about 1 to 3 alpha particle emissions must penetrate
20 through the nucleus of a cell to kill the cell. If the three-dimensional geometry of cells is considered, about 25 alpha-particle emissions are needed per single cell to achieve complete cell killing in a tumor mass with uniform labeling of the cell surface by a radiolabeled protein (see 4th Int. Radiopharmaceutical Dosimetry Symposium, CONF-851113, pp 26-36, 1985). Many alpha-emitting radionuclides
25 are well known in the art, and may be utilized within the present invention. A representative list is presented in Figure 1. Preferred alpha-emitting radionuclides include lead-212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, polonium-210, astatine-211, radium-223, radium-224, and actinium-225.

Particularly preferred alpha-emitters are radium-223 (half-life = 11.4 days) and actinium-225 (half-life = 10.0 days). Radium-223 is a member of the natural uranium-235 decay series (see Figure 2). It exists naturally

in all soils containing uranium and daughter products, but may be found at higher concentrations in uranium mill tailing piles. It may be removed by chemical separation from tailing sands by recovering its predecessor actinium-227.

Briefly, actinium-227 decays naturally to Th-227, which decays naturally to Ra-223 (see Figure 2). Radium-223 may be separated chemically from both Ac-227 and Th-227 by, for example, passing a saline solution over an ion-exchange resin containing the parent radionuclides. The purified salt radium-223 may thus be eluted from the column (see Pilger, UCRL-3877, 1957, University of California Radiation Laboratory, Berkeley, California; Müller, "Präparative Arbeiten über Ac-227 und seine Folgeprodukte, *Sonderdruck aus Radiochimica Acta* 9:181-186, 1968; and Atcher et al., "A Radionuclide Generator for the Production of Pb-211 and its Daughters," *J. Radioanal. Nucl. Chem. (Letters)* 135(3):215-221, 1989). Radium-223 may also similarly be separated from enriched U-235 stockpiles in which natural radioactive decay has allowed the build-up of Ac-227.

An alternative method of producing radium-223 for medical applications is to start with natural radium-226. Within this method, radium-226 is first irradiated in a nuclear reactor to produce radium-227. The radium-227 then beta decays to actinium-227. For example, 1.0 curies of radium-226 is irradiated for about 120 days in a hydride assembly (such as the Fast Flux Test Facility, Richland, Washington). This assembly produces neutrons of epithermal energy, optimum for conversion of Ra-226 to Ra-227, which then beta-decays to Ac-227. Other nuclear reactors may, however, also be used to activate Ra-226 to Ra-227. This procedure produces about 9.5 curies of actinium-227. Radium-223 may then be chemically separated from actinium-227 and thorium-227 utilizing methods described above.

The growth factor may be conjugated to the alpha-emitting radionuclide by various methods, although it is particularly preferred to bind the alpha-emitting radionuclide to a sequestering agent, for example by positioning the alpha-emitting radionuclide within the sequestering agent, which is in turn coupled by a linker to the growth factor. A variety of diverse organic macrocyclic complexing agents may be used to sequester the alpha-emitting radionuclide including, among others, the following groups: (1) spherands, (2) cryptaspherands, (3) cryptands, (4) hemispherands, (5) corrands (modified crown ethers), and (6) podands (acyclic hosts) (see Cram, *Science* 240:760-67, 1988). In general, these macrocyclic ring compounds are large, somewhat spherical organic compounds which resemble cage structures, and have the ability to hold a heavy radionuclide

as a ligand holds a metal ion. The sequestering agent should be selected such that it has both a high affinity and specificity for the alpha-emitting radionuclide as well as a low intrinsic mammalian toxicity. High specificity is essential to avoid displacement by other divalent cations (Mg^{+2} and Ca^{+2}) that are prevalent in physiological fluids. Additionally, the compound should either contain a functional group, or have chemistry which is compatible with the introduction of an appropriate functional group, to allow attachment to the linker.

The affinity of the sequestering agent for the alpha-emitting radionuclide is defined by the system energetics as described by Cram (*supra*). More specifically, as inferred by X-ray crystallographic data of complexed and non-complexed crown ethers, it is believed that the solution conformations of non-complexed ethers lack well-defined cavities with the associated convergently aligned binding sites. During the process of complexation, the crown ether undergoes desolvation and reordering of structure, a process which requires energy. If the sequestering agent presents a rigid prestructured and desolvated cavity to the ion (as is the case for spherands), the energy normally consumed by desolvation and reorganization is reflected in a larger binding constant for the ion. Based on this fundamental principle of reorganization, Cram lists the affinity of hosts for their most complimentary guests as: spherands > cryptaspherands > cryptands > hemispherands > corrands > podands. The difference in binding affinity between spherands and podands is dramatic, for example, the binding constant of a lithium sequestering spherand was found to be 10^{12} higher than its corresponding open-chain podand (*see* Cram, *supra*). Thus, although many different sequestering agents may be utilized within the context of the present invention, spherands which are designed and synthesized specifically to sequester radium-223 are particularly preferred.

Particularly preferred sequestering agents include 18-crown-6 or 21-crown-7 ethers, including for example modified crown ethers such as dicyclohexano-21-crown-7 (Case and McDowell, *Radioact. Radiochem.* 1:58, 1990; McDowell et al., *Solvent Extr. Ion Exch.* 7:377, 1989; for other crown ethers or macrocyclic polyethers, see Pedersen, *Science* 241:536-540, 1988, U.S. Patent No. 4,943,375, Eia et al., *Heterocycles* 32(4):711-722, 1991; Wai and Du, *Anal. Chem.* 62(21):2412-14, 1990; Tang and Wai, *Analyst(London)* 114(4):451-453, 1989). Briefly, Ra^{2+} is bound by the etherate oxygen network comprising the interior cavity of the spherical crown-ether molecule. This binding is believed to be pH dependent: Ra^{2+} complexes with a combination of a proton and smaller Group IA ions for the binding site within the crown cavity. These crown ethers may

additionally be modified with polarizable functional groups (similar to changes made with *closo*- and *nido*-carboarnyl species used in boron-neutron capture therapy), resulting in compounds with greater solubility in aqueous media (see generally, Mizusawa et al., *Inorg. Chem.* 24:1911, 1985). Such changes improve 5 retention of biological specificity after conjugation, and improve the conjugate loading capability of the biological agent. These modifications may be accomplished in tandem with the synthesis of the above-noted crown ethers under appropriate conditions for mild conjugation to the biological delivery system.

Additional crown ethers suitable for use within the present 10 invention may be synthesized, or purchased from various sources including, among others, Aldrich Chemical Co. (Milwaukee, Wis.), Fluka Chemical Corp. (Ronkonkoma, N.Y.), and Nisso Research Chemicals, (Iwai Co. Ltd., Tokyo, Japan). Sequestration of the alpha-emitting radionuclide may be achieved by mixing the sequestering agent with a salt of the alpha-emitting radionuclide which 15 has been dissolved in solvent. The particular solvent chosen depends of course on the solubility of the sequestering agent and alpha-emitting radionuclide. For example, Cram and co-workers prepared the sodium complex of a spherand simply by adding excess salt dissolved in acetonitrile to a methylene chloride solution of the spherand (see Cram and Lein, *J. Am. Chem. Soc.* 107:3657-3668, 20 1985).

The ability of the crown ether to sequester or complex with the alpha-emitting radionuclide may be readily determined (see Cox et al., "Rates and Equilibria of Alkaline-Earth-Metal Complexes with Diaza Crown Ethers in Methanol," *Inorg. Chem.*, 27:4018-4021, 1988; see also Mohite and Khopkar, 25 "Separation of Barium From Alkaline Earths and Associated Elements by Extraction with Dibenzo-18-crown-6 From a Picrate Medium," *Analytica Chimica Acta*, 206:363-367, 1988). Briefly, separation of the complexed and free radionuclide can be accomplished by partitioning between an organic solvent (such as chloroform) and water. The complexed radionuclide will partition into 30 the organic phase, whereas the free radionuclide will reside exclusively in the aqueous phase. Alternatively, a variety of chromatographic techniques such as High Performance Liquid Chromatography (HPLC) or Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) may be utilized to separate sequestered radionuclide from the free cation. Once isolated, verification of the 35 molecular architecture may be accomplished. Briefly, the mode of cation binding can take two forms: (1) through external association (*i.e.*, anion/cation pairing without bond formation), or (2) via coordination of the cation to the crown-ether

oxygen network. Specificity and strong binding, which are preferred for the present applications, are dependent on the latter type of association. Single crystal X-ray diffraction techniques may be used to unambiguously assign the type of interaction for the solid materials, and ^{17}O , ^{13}C and $^1\text{H-NMR}$ may be used to 5 determine the structures of target materials in solution.

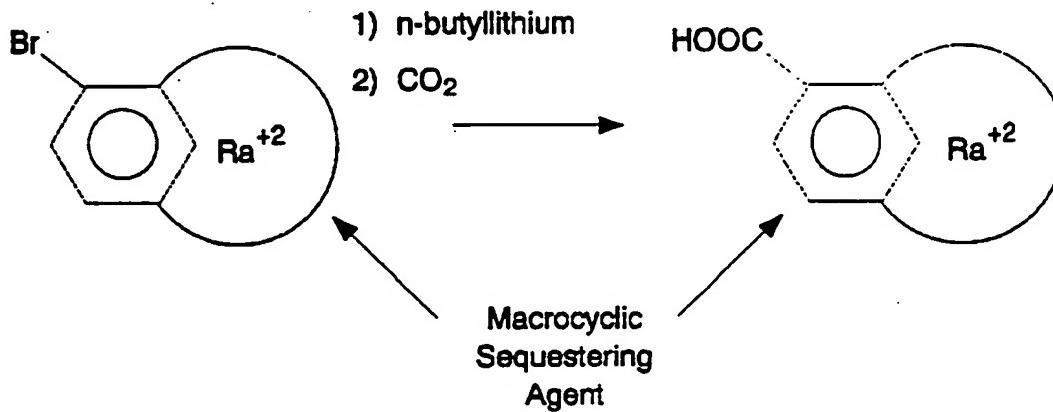
As noted above, within one embodiment of the present invention the alpha-emitting radionuclide is positioned within a sequestering agent which is in turn coupled by a linker to preferably either the amino ("N") or carboxy ("C") terminus of the growth factor. The linker serves to place an inert "spacer" 10 between the biologically active growth factor and the alpha-emitting radionuclide containing complex. This space minimizes steric interactions that may interfere with the growth factor's affinity towards its target. The optimum length of the spacer arm is primarily dependent on the affinity of the growth factor for its target receptor. The higher this affinity, the smaller the relative importance of stearic 15 repulsion between the sequestering agent and the target receptors. A virtually limitless number of linkers may be selected which are suitable for use within the present invention, although presently preferred linkers include disulfides, dicarboxylic acids, polycarbon chains, and modified polycarbon chains. Preferred linkers include hydrocarbon chains which range in length from 4 to 18 carbon 20 atoms. Particularly preferred linkers have at least six methylene units such as hexamethylene diamine.

The linker may be attached to any of a number of extraanular functionalities on the sequestering agent, although carboxy and amino functionalities are particularly preferred. Within one aspect of the invention, if 25 the extraanular functionalization is a carboxy group, then a first synthetic step could involve reaction of the sequestering agent with hexamethylene diamine. Subsequent reaction with the C-terminus of the growth factor would complete synthesis of the conjugate. Alternatively, as noted above, the linker may be coupled to other aspects of the growth factor such as the N-terminus. Within this 30 embodiment, after reaction with hexamethylene diamine the sequestering agent may be reacted with succinic anhydride. Subsequent coupling of the linker to the growth factor may then be accomplished through the N-terminus of the growth factor.

Alternatively, within another aspect of the present invention, the 35 sequestering agent may contain an amino functionality. In these cases, a dicarboxylic acid linker (for example, octanedioic acid) may be utilized to couple the sequestering agent to the N-terminus of the growth factor. On the other hand,

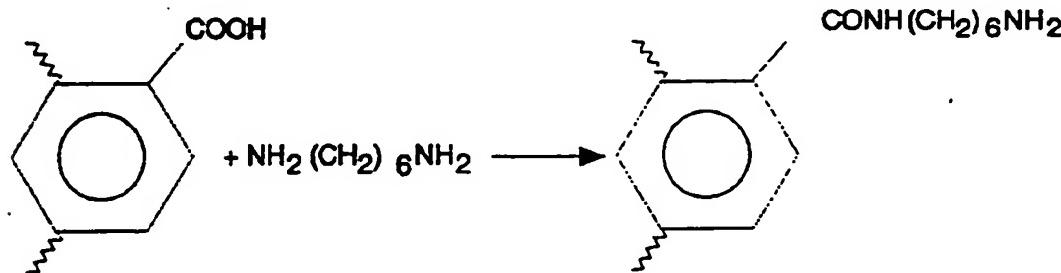
if the sequestering agent is reacted with ethylene diamine after condensation with the dicarboxylic acid, linkage to the growth factor may be accomplished through the C-terminus.

Within one embodiment of the present invention, in order to allow the covalent attachment of the sequestering agent to the linker an appropriate functionality is inserted into the sequestering agent. For example, a bromine atom may be incorporated into the appropriate position of an aromatic constituent during synthesis of the macrocyclic compound (see Skowronska-Ptasinska et al., *J. Org. Chem.* 53:5484-91, 1988). Sequential treatment of this compound with n-butyllithium and CO₂ yields the carboxy analog:

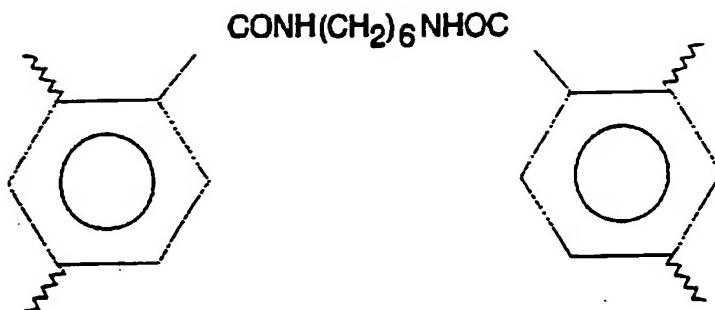


It should be noted, however, that synthetic reactions leading to these types of sequestering agents may produce very low yields.

Since the growth factor is likely to be the limiting reactant, the next step within this embodiment of the invention is the reaction between the functionalized sequestering agent and the linker:



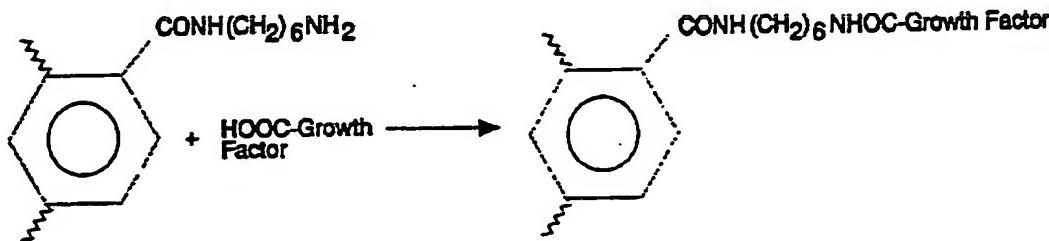
If the sequestering agent is not immobilized on a rigid support the following by-product may also be produced:



5

Thus, chromatographic purification of the reaction mixture to isolate the desired product may be necessary before proceeding. Briefly, standard semi-preparative chromatographic separations based upon, for example, RP-HPLC or HPLC purification, may be utilized to purify the target compounds from 10 the synthetic mixtures. Products may be detected either by refractive index or by the more sensitive technique of ultraviolet adsorption detection. Within one embodiment, a chromophoric benzene moiety is incorporated into the sequestering agent to facilitate detection during chromatographic purification.

The final reaction within this embodiment involves a similar 15 reaction between the sequestering agent-linker (organic soluble) and the carboxy terminus of the growth factor (water soluble) as summarized below:



20

Solubility incompatibilities may be overcome by use of a 50:50 dimethylformamide:water solvent system (see generally Cooper, *The Tools of Biochemistry*, Wiley, New York, pp. 234-255, 1977; Cuatrecasas, "Protein Purification by Affinity Chromatography on Polyacrylamide Beads," *J. Biol. Chem.* 245:3059, 1970; and Cuatrecasas, "Affinity Chromatography of Macromolecules," in *Advances in Enzymology*, A. Meister (ed.), Wiley, New York, p. 29, 1972).

Within another aspect of the present invention, the growth factor is conjugated to non-radioactive iodine. Briefly, non-radioactive iodine may be obtained from many commercial sources, including, for example, Sigma Chemical Co. (St. Louis, Mo.). Various methods which are typically used to label proteins 5 with radioactive iodine may also be utilized to conjugate non-radioactive iodine to the growth factor. For example, iodide (normally supplied as NaI) may be oxidized to form I₂, which then attacks tyrosyl and histidyl side chains. Representative methods utilizing this technique include the Chloramine T method (Hunter and Greenwood, *Nature* 194:495-496, 1962), the Iodogen method (see 10 Fraker and Speck, *Biochem. Biophys. Res. Commun.* 80:849-857, 1978), and the lactoperoxidase method (see Hubbard and Cohn, *J. Cell Biol.* 55:290-405, 1972). Alternatively, an iodinated reagent containing a reactive coupling group may be bound to the protein (see Bolton and Hunter, *Biochem. J.* 133:529-539, 1973).

Within other aspects of the invention, numerous additional growth 15 factor conjugates are provided. Within one embodiment, these growth factor conjugates comprise a growth factor, and a radioactive isotope which emits gamma radiation. Representative examples of such radioactive isotopes include rhenium-186, technetium-99m, iodine-131, selenium-75, iodine-123, iodine-125, iodine-124, indium-111, copper-67, radium-223, gold-198, yttrium-90, chromium- 20 51, iron-52, copper-64, gallium-67, gallium-66, gallium-72, gallium-68, zirconium-89, ruthenium-97, lead-203, rhodium-105, rhenium-188, gold-199, astatine-211, bromine-76, bromine-77, fluorine-18, bismuth-206, mercury-197, and mercury-203. Within other embodiments of the invention, the growth factor conjugate comprises a growth factor and a cytotoxic agent. Representative examples of 25 cytotoxic agents include (in addition to the various alpha and gamma emitters discussed above) oxyanions of a metal selected from the group consisting of manganese, technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium.

Conjugated growth factors of the present invention may additionally 30 be purified utilizing a variety of techniques, including among others, column chromatography, HPLC, and RP-HPLC.

Conjugates of the present invention may be utilized in various ways. For example, they may be employed in *in vitro* assays as described below in order to kill specific cells.

35 Additionally, as noted above, the conjugates of the present invention may be utilized for the treatment and detection of cancer in warm-blooded animals. Many warm-blooded animals may be treated and diagnosed for

cancer, including for example, mice, rats, sheep, cows, pigs, monkeys, and humans. Briefly, as noted above, within one aspect of the present invention a method for treating cancer in warm-blooded animals is provided, comprising the step of administering to the animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, the growth factor conjugate being capable of specifically binding to a defined population of cancer cells. Within one embodiment, the alpha-emitting radionuclide is selected from the group consisting of lead 212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, polonium-223, radium-224, and actinium-225.

Within another aspect of the present invention, a method for treating cancer in warm-blooded animals is provided, comprising administering to the animal an effective amount of a conjugate of a growth factor and yttrium-90, the growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

Within yet another aspect of the present invention, a method for treating cancer in warm-blooded animals is provided, comprising the step of administering to the animal an effective amount of a conjugate of a growth factor and an oxyanion of a metal selected from the group consisting of manganese, technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium, the growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

Within another aspect of the present invention, a method for treating cancer in warm-blooded animals is provided, comprising the step of administering to the animal an effective amount of a conjugate of a growth factor and non-radioactive iodine, the growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

Within particularly preferred embodiments of the invention, the present invention provides, prior to the step of administering an effective amount of a conjugate as described above, administering an unlabeled growth factor capable of specifically binding to the defined population of cancer cells, in an amount sufficient to mask growth factor receptors in healthy tissues of the animal. Briefly, in order to concentrate the radioisotope preferentially in cancer cells and avoid excessive damage to normal cells, administration of the conjugated growth factor is preceded by the step of administering a "cold" or unlabeled growth factor capable of binding to growth factor receptors in both normal and cancer cells, thereby reducing the number of receptor sites on normal cells available for binding and thus minimizing radiation damage to normal cells. Masking of growth

factor receptors may be accomplished in methods for both treating and diagnosing cancer, as described herein.

Within one aspect of the present invention, a method for detecting cancer is provided, comprising the steps of (a) administering to a warm-blooded animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, the growth factor being capable of specifically binding to a defined population of cancer cells, and (b) detecting the presence of the conjugate within the warm-blooded animal, and therefrom determining the presence of cancer. Briefly, conjugates or pharmaceutical compositions as described above 5 may be administered in an effective amount as determined by experimental trials. The presence of the conjugate may be detected by any suitable nuclear medicine radiation camera which detects the requisite particle emissions (e.g., alpha or gamma). In the case of alpha emitters, a Nuclear Medicine Anger camera fitted with a collimator for the Tc^{99m} energy window is particularly preferred.

10 Within another aspect of the present invention, a method for detecting the presence of cancer in warm-blooded animals is provided comprising the steps of (a) administering to the warm-blooded animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, the growth factor conjugate being capable of specifically binding to a defined population of 15 cancer cells, and (b) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of cancer.

20 Within yet another aspect of the present invention a method for detecting the presence of cancer in warm-blooded animals is provided, comprising the steps of (a) administering to the animal an unlabeled growth factor capable of 25 specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of a conjugate of the growth factor and a radioactive isotope which emits gamma radiation, and (c) detecting the presence and location of the conjugate within the warm-blooded animal and 30 therefrom determining the presence of cancer. Within various embodiments of the present invention, the radioactive isotope is selected from the group consisting of rhenium-186, technetium-99m, iodine-131, selenium-75, iodine-123, iodine-125, iodine-124, indium-111, copper-67, radium-223, gold-198, yttrium-90, chromium-51, iron-52, copper-64, gallium-67, gallium-66, gallium-72, gallium-68, zirconium-35 89, ruthenium-97, lead-203, rhodium-105, rhenium-188, gold-199, astatine-211, bromine-76, bromine-77, fluorine-18, bismuth-206, mercury-197, and mercury-203.

Within another aspect of the present invention, a method for diagnosing and treating cancer in warm-blooded animals is provided, comprising (a) administering to the animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask 5 growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of conjugate of the growth factor and a radioactive isotope which emits gamma radiation, (c) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of the cancer, and (d) administering an effective amount of a second 10 conjugate of a growth factor and a radioactive isotope or non-radioactive iodine, such that the cancer is treated.

Within yet another aspect of the present invention, a method for diagnosing and treating cancer in warm-blooded animals is provided, comprising the steps of (a) administering to the animal an unlabeled growth factor capable of 15 specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of the animal, (b) administering to the animal an effective amount of a first conjugate of a growth factor and a radioactive isotope which emits gamma radiation, (c) detecting the presence and location of the conjugate within the warm-blooded animal and 20 therefrom determining the presence of the cancer, and (d) administering an effective amount of a second conjugate of a growth factor and a cytotoxic metal ion, such that the cancer is treated.

Within various embodiments of the invention, the cytotoxic agent is an oxyanion of a metal selected from the group consisting of manganese, 25 technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium. Within yet other embodiments of the invention, the cytotoxic agent is an alpha particle emitting radioactive isotope selected from the group consisting of lead-212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, radium-224, and actinium-225.

30 Within a further embodiment of the invention, pharmaceutical compositions are provided. Briefly, representative examples of pharmaceutical compositions include a conjugate of a growth factor and an alpha-emitting radionuclide, or a conjugate of a growth factor and non-radioactive iodine, or any of the other growth factor conjugates discussed above, along with a 35 pharmaceutically acceptable carrier or diluent. Suitable pharmaceutically acceptable carriers or diluents include neutral buffered saline or saline. Additionally, the pharmaceutical composition may contain other constituents,

including for example buffers, carbohydrates such as glucose, sucrose, or dextrose, preservatives, as well as other stabilizers or excipients. Although appropriate dosages may be determined by experimental trials, about 5×10^{10} to 5×10^{11} conjugate complexes/70kg of adult weight may be administered assuming a 1:1
5 ratio of growth factor to the alpha-emitter or non-radioactive iodine. Nevertheless, the amount and frequency of administration will depend of course on many factors such as the condition of the patient, the nature and severity of the disease, as well as the type of cancer being treated. In addition, as discussed above, it is generally preferable to first mask growth factor receptors with
10 unlabeled growth factor, in order to minimize damage to normal healthy tissues.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

EFFECTS OF ^{131}I RADIOLABELED EGF ON A431 CELLS

5

A. Preparation of Cells

The human cervical epidermoid carcinoma cell line A431 (available from the American Type Culture Collection or "ATCC," Rockville, Maryland, under accession number CRL 1555) was grown in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum. The cells were harvested by trypsinization with 0.05% trypsin and counted with trypan blue to obtain the number of live cells. A431 cells have approximately $1\text{-}2 \times 10^6$ EGF receptors per cell.

B. Radioiodination of Epidermal Growth Factor

One hundred micrograms of murine EGF (GIBCO Laboratories/Life Technologies, Inc., Grand Island, N.Y.) was radiolabeled by iodination with ^{131}I (Dupont, Wilmington, Del.) using a modified method of lactoperoxidase procedure as described by Leung et al. (*Proc. Soc. Exp. Biol. Med.*, 196(4):385-9, 1991) which modifies the procedure of Thorell and Johansson (*Biochim. Biophys. Acta* 251:363 1971). Briefly, H_2O_2 was added in four or five aliquots at 1-min intervals to a reaction mixture of 10 mCi of ^{131}I , 100 μg of EGF, and 100 μg of lactoperoxidase. The labeled EGF was then separated from the free $[^{131}\text{I}]$ NaI and lactoperoxidase by gel filtration on a Sephadryl S-200 column (1 x 30 cm) that was previously equilibrated with 0.05 M phosphate-buffered saline containing 0.1% bovine serum albumin pH 7.6). Figure 3 shows the iodination profile of the ^{131}I to EGF, demonstrating that >90% of the ^{131}I was labeled into the EGF molecule.

C. Cytotoxicity Assay

A431 cells were grown in 6 well cultured plates. Two of the wells were exposed to approximately 500 μCi of radiolabeled EGF (^{131}I -EGF), two of the wells were exposed to free ^{131}I similar to the quantity of ^{131}I -EGF, and the other two wells of A431 cells were exposed to unlabeled EGF similar to the quantity of ^{131}I -EGF. The A431 cells were exposed to the three different treatments for one hour. Cells were washed twice with PBS buffer, and fed with DMEM and cultured for 5 days. The experiment was repeated in four replicate of

6 well plates. At the end of day 5, cells were harvested and counted. Figure 3 shows that the wells of A431 cells exposed to ^{131}I -EGF have significantly fewer viable cells as compared to cells exposed to free ^{131}I or cells exposed to unlabeled EGF. Thus, radiolabeled growth factor can be used as a specific cytotoxic agent 5 for human tumor cells which possess high numbers of the growth factor receptor.

EXAMPLE 2

EFFECTS OF NON-RADIOACTIVE IODINE LABELED EGF ON A431 AND L CELLS.

10 A. Preparation of Cells

A431 cells and L cells were prepared as described above in Example 1. L cells are a murine fibroblast cell line which is available from the ATCC under accession number CRL 6362. Unlike A431 cells, L cells have less than 1000 EGF receptors per cell. Cells were grown and harvested as described above in 15 Example 1A.

B. Iodination of Epidermal Growth Factor

Epidermal Growth Factor was iodinated utilizing a procedure identical to that described in Example 1B above, except that non-radioactive 20 iodine (Sigma Chemical Co., St. Louis, Mo.) was utilized in place of radioactive iodine.

C. Cytotoxicity Assay

Cells were prepared and analyzed essentially as described in 25 Example 1C. As illustrated in Figures 4 through 7, ^{131}I -EGF has a cytotoxic effect on A431 cells (see Figure 4), but not on cells with low numbers of receptors such as L cells (see Figure 6). When experiments were performed with EGF labeled with non-radioactive iodine, there was a surprising cytotoxic effect similar to that of EGF labeled ^{131}I . Furthermore, the cytotoxic effect did not extend to L 30 cells, indicating that the toxic effect was mediated by EGF binding to the cell.

EXAMPLE 3

MASKING OF EGF-GROWTH FACTOR RECEPTORS PRIOR TO ADMINISTRATION OF ^{123}I -EGF

35

In order to determine the biodistribution of ^{123}I EGF, the following experiment was undertaken. Briefly, approximately 1×10^6 A431 cells were

injected subcutaneously into nude mice. The cells were allowed to grow in the mice for one to two weeks, after which the mice were injected either with or without unlabeled EGF, followed by the injection of ^{123}I -EGF. The mice were then sacrificed and the percent of injected dose per gram determined in the blood, tumor, muscle, lung, kidney, spleen, liver, intestine, thyroid, urine and stomach.

5 tumor, muscle, lung, kidney, spleen, liver, intestine, thyroid, urine and stomach.
 The results of this experiment are set forth below in Tables I and II.

TABLE I

10 **A-431 With Labeled EGF (I-123) Biodistribution**
Summary of Percent Injected Dose Per Gram

MOUSE	TISSUE										
	Blood	Tumor	Muscle	Lung	Kidney	Spleen	Liver	Intestine	Thyroid	Urine	Stomach
normal	3.2	--	0.6	2.3	14.6	1.7	35.8	0.8	0.0	36.4	2.1
25	1-1	5.8	3.5	0.6	2.8	25.9	2.1	33.1	0.7	3.5	2.7
	1-2	5.1	2.6	0.6	2.8	24.5	2.4	43.9	0.3	3.2	--
	1-3	2.8	1.3	0.3	1.8	16.6	1.4	6.0	1.9	2.0	--
	1-4	4.1	2.7	0.7	2.5	22.0	1.8	14.9	0.6	3.0	2.6
30	average	4.5	2.5	2.2	2.5	22.3	1.9	24.5	0.9	2.9	2.7
	5-1	2.8	4.7	0.7	2.8	16.6	2.2	24.1	0.6	3.9	75.1
	5-2	2.5	2.8	0.6	2.6	14.2	1.9	28.2	0.3	5.0	24.3
	5-3	4.7	3.9	1.2	4.6	17.8	3.1	22.0	1.3	7.4	39.2
	5-4	4.7	3.9	1.2	4.6	17.8	3.1	22.0	1.3	7.4	39.2
35	average	3.2	3.6	0.8	3.2	15.6	2.3	25.5	0.8	5.1	46.0
	50-3-1	18.6	4.0	1.2	8.4	30.5	5.4	6.7	3.8	6.5	--
	50-3-2	20.9	1.7	1.3	9.8	23.1	6.6	6.3	4.8	7.0	--
40	average	19.8	2.9	1.3	9.1	26.8	6.0	6.5	4.2	6.3	--
	25-10-1	10.1	2.1	1.3	6.8	48.6	3.2	7.4	2.6	3.5	--
	25-14-1	0.0	0.4	0.0	0.1	0.1	0.0	0.0	0.0	0.3	1.5
	50-14-1	0.0	0.3	0.0	0.1	0.1	0.0	0.0	0.0	0.2	1.3
45	0.6										

TABLE II

A-431 With Labeled EGF (I-123) Biodistribution
Summary of Tissue to Blood Ratios

5

	MOUSE	TISSUE									
		Tumor	Muscle	Lung	Kidney	Spleen	Liver	Intestine	Thyroid	Urine	Stomach
10	normal	---	0.2	0.7	4.6	0.5	11.2	0.3	0.0	11.4	0.7
15	1-1	0.6	0.1	0.5	4.5	0.4	5.7	0.1	0.6	0.5	0.2
	1-2	0.5	0.1	0.5	4.8	0.5	8.6	0.1	0.6	---	0.3
	1-3	0.5	0.1	0.6	5.8	0.5	2.1	0.7	0.7	---	0.2
20	1-4	0.7	0.2	0.6	5.4	0.4	3.6	0.1	0.7	0.6	0.2
	average	0.6	0.1	0.6	5.1	0.5	4.0	0.3	0.7	0.6	0.2
25	5-1	1.6	0.2	1.0	5.9	0.8	8.5	0.2	1.4	26.6	2.8
	5-2	1.1	0.2	1.0	4.7	0.7	9.5	0.3	1.4	15.6	2.4
	5-3	1.0	0.2	1.0	4.7	0.7	9.5	0.3	1.4	15.6	2.4
	5-4	0.8	0.3	1.0	3.8	0.7	4.7	0.3	1.6	8.4	3.3
	average	1.1	0.2	1.0	5.0	0.8	8.5	0.2	1.6	15.1	2.9
30	50-3-1	0.2	0.1	0.5	1.6	0.3	0.4	0.2	0.4	---	0.2
	50-3-2	0.1	0.1	0.5	1.1	0.3	0.3	0.2	0.3	---	0.2
	average	0.2	0.1	0.5	1.4	0.3	0.4	0.2	0.4	---	0.2
35	25-10-1	0.2	0.1	0.7	4.8	0.3	0.7	0.3	0.3	---	0.4
	25-14-1	14.7	0.2	2.7	3.7	1.2	1.7	1.4	10.9	58.9	14.5
40	50-14-1	9.2	0.2	1.8	3.6	0.8	1.2	1.0	7.5	38.2	18.5

- Notes:
- 1. Normal mouse sacrificed at 3 minutes.
 - 2. Group 1 mice sacrificed at 3 minutes.
 - 3. Group 5 mice sacrificed at 48 minutes.
 - 4. Mice 50-3-1 and 2 were blocked with 50 ug native EGF 3 minutes prior to labeled EGF; sacrificed at 3 minutes.
 - 5. Mouse 25-10-1 was blocked with 25 ug native EGF 3 minutes prior to labeled EGF; sacrificed at 10 minutes
 - 6. Mice 25-14-1 and 50-14-1 were injected with 25 ug and 50 ug native EGF respectively, 3 minutes prior to labeled EGF; sacrificed at 14 hours.

As shown in Table II, administration of unlabeled growth factor sufficient to mask growth factor receptors in normal healthy tissue, results in the more specific targeting of ^{123}I -EGF.

5 From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Furthermore, various references have been cited herein which provide additional detail and experimental insight, and are therefore
10 hereby incorporated by reference. Accordingly, the invention is not limited except as by the appended claims.

While the applicant has disclosed the invention in the context of diagnosis and treatment of cancer, it is believed that the invention in its broadest aspect is applicable to the delivery of therapeutic agents to treat other disease
15 conditions; for example, use of growth factor conjugated to a therapeutic agent such as an antibiotic would enhance the efficacy of the therapeutic agent for treating diseases such as rheumatoid arthritis.

Claims

1. A conjugate of a growth factor and an alpha-emitting radionuclide, said growth factor being capable of specifically binding to a defined population of cancer cells.
2. The conjugate of claim 1 wherein said growth factor is coupled to said alpha-emitting radionuclide by a linker.
3. The conjugate of claim 1 wherein said alpha-emitting radionuclide is bound to a sequestering agent.
4. The conjugate of claim 3 wherein the sequestering agent is a macrocyclic complexing agent.
5. The conjugate of claim 3 wherein said sequestering agent is a crown ether.
6. The conjugate of claim 5 wherein said crown ether is selected from the group consisting of 21-crown-7 ethers and 18-crown-6 ethers.
7. The conjugate of claim 2 wherein said linker is a polycarbon compound.
8. The conjugate of claim 2 wherein said linker is selected from the group consisting of disulfides, dicarboxylic acids, and polycarbons.
9. The conjugate of claim 2 wherein said linker is hexamethylene diamine.
10. The conjugate of claim 2 wherein said linker is coupled to a portion of the growth factor selected from the group consisting of the N-terminus and the C-terminus.
11. The conjugate of claim 1 wherein the alpha-emitting radionuclide is selected from the group consisting of lead-212/bismuth-212, bismuth-213/polonium-

213, bismuth-212m, bismuth-212, polonium-206, polonium-210, astatine-211, radium-223, radium-224, and actinium-225.

12. A conjugate of a growth factor and non-radioactive iodine, said growth factor being capable of specifically binding to a defined population of cancer cells.

13. The conjugate of claims 1 or 12 wherein said growth factor is selected from the group consisting of epidermal growth factor, transforming growth factor - alpha, fibroblast growth factors, insulin like growth factor I and II, and nerve growth factor.

14. A pharmaceutical composition comprising a conjugate of a growth factor and an alpha-emitting radionuclide, and a pharmaceutically acceptable carrier or diluent, said growth factor being capable of specifically binding to a defined population of cancer cells.

15. A pharmaceutical composition comprising a conjugate of a growth factor and non-radioactive iodine, and a pharmaceutically acceptable carrier or diluent, said growth factor being capable of specifically binding to a defined population of cancer cells.

16. The pharmaceutical composition of claims 14 or 15 wherein said growth factor is selected from the group consisting of epidermal growth factor, transforming growth factor - alpha, fibroblast growth factors, insulin like growth factor I and II, and nerve growth factor.

17. A method for treating cancer in warm-blooded animals comprising administering to said animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

18. The method of claim 17, wherein said alpha-emitting radionuclide is selected from the group consisting of lead 212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, polonium-223, radium-224, and actinium-225.

19. A method for treating cancer in warm-blooded animals, comprising administering to said animal an effective amount of a conjugate of a growth factor and yttrium-90, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

20. A method for treating cancer in warm-blooded animals, comprising administering to said animal an effective amount of a conjugate of a growth factor and an oxyanion of a metal selected from the group consisting of manganese, technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

21. A method for treating cancer in warm-blooded animals comprising administering to said animal an effective amount of a conjugate of a growth factor and non-radioactive iodine, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells.

22. The method of claims 16 - 21, further comprising, prior to the step of administering an effective amount of a conjugate, administering an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptors in healthy tissues of said animal.

23. A method for detecting the presence of cancer in warm-blooded animals, comprising:

- (a) administering to said animal an effective amount of a conjugate of a growth factor and an alpha-emitting radionuclide, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells; and
- (b) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of cancer.

24. A method for detecting the presence of cancer in warm-blooded animals, comprising:

- (a) administering to said animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of said animal;
- (b) administering to said animal an effective amount of a conjugate of said growth factor and a radioactive isotope which emits gamma radiation; and

(c) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of cancer.

25. The method of claim 24 wherein said radioactive isotope is selected from the group consisting of rhenium-186, technetium-99m, iodine-131, selenium-75, iodine-123, iodine-125, iodine-124, indium-111, copper-67, radium-223, gold-198, yttrium-90, chromium-51, iron-52, copper-64, gallium-67, gallium-66, gallium-72, gallium-68, zirconium-89, ruthenium-97, lead-203, rhodium-105, rhenium-188, gold-199, astatine-211, bromine-76, bromine-77, fluorine-18, bismuth-206, mercury-197, and mercury-203.

26. A method for diagnosing and treating cancer in warm-blooded animals, comprising:

(a) administering to said animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of said animal;

(b) administering to said animal an effective amount of conjugate of said growth factor and a radioactive isotope which emits gamma radiation;

(c) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of said cancer; and

(d) administering an effective amount of a second conjugate of a growth factor and a radioactive isotope or non-radioactive iodine, such that said cancer is treated.

27. A method for diagnosing and treating cancer in warm-blooded animals, comprising:

(a) administering to said animal an unlabeled growth factor capable of specifically binding to a defined population of cancer cells, in an amount sufficient to mask growth factor receptor sites in healthy tissues of said animal;

(b) administering to said animal an effective amount of a first conjugate of a growth factor and a radioactive isotope which emits gamma radiation;

(c) detecting the presence and location of the conjugate within the warm-blooded animal and therefrom determining the presence of said cancer; and

(d) administering an effective amount of a second conjugate of a growth factor and a cytotoxic agent, such that said cancer is treated.

28. The method of claims 26 - 27 wherein said radioactive isotope is selected from the group consisting of rhenium-186, technetium-99m, iodine-131, selenium-75, iodine-123, iodine-125, iodine-124, indium-111, copper-67, radium-223, gold-198, yttrium-90, chromium-51, iron-52, copper-64, gallium-67, gallium-66, gallium-72, gallium-68, zirconium-89, ruthenium-97, lead-203, rhodium-105, rhenium-188, gold-199, astatine-211, bromine-76, bromine-77, fluorine-18, bismuth-206, mercury-197, and mercury-203.

29. The method of claim 27 wherein said cytotoxic agent is an oxyanion of a metal selected from the group consisting of manganese, technetium, rhenium, chromium, molybdenum, tungsten, vanadium, and tellurium.

30. The method of claim 27 wherein said cytotoxic agent is an alpha particle emitting radioactive isotope selected from the group consisting of lead-212/bismuth-212, bismuth-213/polonium-213, bismuth-212m, bismuth-212, polonium-206, radium-224, and actinium-225.

31. A composition according to claims 1-12 for use as an active therapeutic substance.

32. A composition according to claim 13 for use as an active therapeutic substance.

33. A conjugate of a growth factor and an alpha-emitting radionuclide, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells, for use in a method for treating cancer.

34. A conjugate of a growth factor and non-radioactive iodine, said growth factor conjugate being capable of specifically binding to a defined population of cancer cells, for use in a method for treating cancer.

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	A	B	C	D	E	F	G	H
1	Isotope	Atomic Mass	Half-Life	Alpha	Beta	Gamma	MeV	Particle Me
2	3Li8	8.02	0.844 s	a	b		16.01	12.5
3	3Li9	9.03	0.178 s	35%	b		13.61	0.3
4	4Be8	8.01	0.067 fs	100%	b			0.046
5	4Be11	11.02	13.8 s	a	b		11.48	11.48
6	5B8	8.02	0.772 s	a	b			
7	5B9	9.01	0.85 as	a				
8	5B12	12.01	0.02 s	1.60%	b		13.37	
9	6C9	9.03	127 ms	a	b		16.5	
10	7N12	12.02	11.0 ms	a	b		17.34	16.38
11	7N16	16.01	7.13 s	a	b		10.42	1.7
12	11Na20	20.01	0.446 s	a	b			2.15
13	23V44	43.97	0.09 s	a	b		13.7	
14	52Te108	107.93	2.1 s	68%	b	g		
15	52Te109	108.93	4.2 s	4%	b	g		
16	53I110	109.94	0.65 s	17%	b	g	12	
17	62Sm146	145.91	1.03e8 y	100%				2.5
18	62Sm147	146.91	1.08e11y	100%				2.23
19	62Sm148	147.91	7e15 y	100%				1.96
20	62Sm149	148.92	1.0e16 y	100%				
21	64Gd148	147.92	75 y	100%				3.1828
22	64Gd150	149.92	1.8e6 y	100%				2.73
23	65Tb149	148.92	4.15 hr	16%			3.7	3.97
24	66Dy150	149.93	7.17 m	33%	b	67%	1.8	4.233
25	66Dy151	150.93	17 m	6%	5%	89%	2.76	4.067
26	66Dy154	153.92	3ee6 y	100%				2.87
27	67Ho151m		48 s	13%	b	87%		4.605
28	67Ho151	150.93		20%	b	80%	5.1	4.519
29	67Ho152m		51 s	10%	b	90%		4.453
30	67Ho152	151.93	2.4 m	12%	b	88%	6.4	4.387
31	68Er152	151.93	10.3 s	90%	b	g	3.12	4.804
32	68Er153	152.93	37.1 s	53%	b	g		4.674
33	68Er154	153.93	3.7 m	0.50%	b	g	2.014	4.166
34	69Tm153	152.94	1.6 s	90%	b	g	6.49	5.11
35	69Tm154m		3.4 s	a	b	g		5.03
36	69Tm154	153.94	8.3 s	44%	b	g	7.99	4.96
37	69Tm155	154.94	25 s	a	b	g	5.55	4.45
38	69Tm156m		19 s	100%				4.46
39	69Tm156	155.94	80 s	a	b	g	7.03	4.23
40	69Tm157	156.94	3.6 m	a	b	g	4.63	3.97
41	70Yb154	153.95	0.4 s	93%	b	g		5.32
42	70Yb155	154.95	1.7 s	84%	b	g		5.19
43	70Yb156	155.94	24 s	79%	b	g	3.7	4.69
44	70Yb157	156.94	39 s	0.50%	b	g	5.2	4.69
45	71Lu155	154.95	0.07 s	a		g	7.97	5.66
46	71Lu156m		0.21 s	a	b	g		5.57
47	71LU156	155.95	0.5 s	a	b	g	9.67	5.45

FIGURE 1A

	A	B	C	D	E	F	G	H
48	71Lu157	156.95	5.5 s	6%	b	g	6.99	5
49	71Lu158	157.95	10 s	1%	b	g	8.78	4.67
50	72Hf158	157.95	2.9 s	46%		54%	4.9	5.27
51	72Hf159	158.95	5.6 s	12%	b	g	6.76	5.09
52	72Hf160	159.95	12 s	3%	b	g	4.21	4.78
53	72Hf161	160.95	17 s	100%				4.6
54	73Ta159	158.96	0.6 s	80%	b	g	8.49	5.6
55	73Ta160	159.96		a	b	g	10.3	5.41
56	73Ta161	160.96		a	b	g	7.55	5.15
57	73Ta164	163.95	13.6 s	a	b		8.35	4.62
58	74W160	159.97	0.08 s	100%				5.92
59	74W161	160.97	0.41 s	82%	b	g	8.34	5.78
60	74W162	161.96	1.39 s	46%	b	g	5.72	5.54
61	74W163	162.96	2.8 s	41%	b	g	7.54	5.38
62	74W164	163.96	6 s	3%	b	g	5.08	5.15
63	74W165	164.96	5.1 s	1%	b	g	6.86	4.91
64	74W166	165.96	16 s	1%	b	g	4.42	4.74
65	75Re162	161.98	0.10 s	100%				6.12
66	75Re163	162.97	0.26 s	a	b	g	9.04	5.92
67	75Re164	163.97	0.9 s	a	b	g	10.9	5.78
68	75Re165	164.96	2.4 s	13%	b	g	8.18	5.51
69	75Re166	165.97	2.2 s	a	b	g	9.98	5.5
70	75Re167	166.96	2.0 s	a	b	g	7.52	5.35
71	75Re168	167.96	2.9 s	a	b	g	9.08	5.14
72	760s166	165.97	0.18 s	72%	b	g	6.27	5.98
73	760s167	166.97	0.7 s	24%	b	g	8.11	5.84
74	760s168	167.97	2.2 s	49%	b	g	7.57	
75	760s169	168.97	3.3 s	17%	b	g	5.16	5.57
76	760s170	169.96	7.1+- .2 s	a	b	g	6.78	5.4
77	760s171	170.96	7.9+- .6 s	2%	b	g	6.78	5.24
78	760s172	171.96	19+- 2 s	1%	b	g	4.43	5.1
79	760s173	172.96	16+- 5 s	0.02%	b	g	6.01	4.94
80	760s174	173.96	44+- 4 s	0.02%	b	g	3.67	4.76
81	760s186	185.95	2ee15 y	100%				2.75
82	77Ir170	169.97	1.05+- .1 s	100%				6.03
83	77Ir171	170.97	1.6+- .1 s	100%				5.91
84	77Ir172	171.97	2.1+- .1 s	100%				5.811
85	77Ir173	172.97	3+- .1 s	100%				5.665
86	77Ir174	173.97	4+- 1 s	100%				5.478
87	77Ir175	174.96	4.5+- 1 s	100%				5.393
88	77Ir176	175.96	8+- 1 s	100%				5.118
89	77Ir177	176.96	21+- 2 s	100%				5.011
90	78Pt172	171.98	.01 s	100%				6.31
91	78Pt173	172.98	0.34 s	a	b	g	8.12	6.2
92	78Pt174	173.97	.9+- .01 s	83%	b	g	5.73	6.04
93	78Pt175	174.97	2.52+- .08	35%	b	g	7.43	5.831
94	78Pt176	175.97	6.3+- .1 s	40%	b	g	5.08	5.528

FIGURE 1B

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	A	B	C	D	E	F	G	H
95	78Pt177	176.97	11+-2 s	9%		91%	6	53
96	78Pt178	177.97	21+-7 s	7%		93%	4.34	5.286
97	78Pt179	178.97	43 s	a	b	g	5.66	5.16
98	78Pt180	179.96	52+-3 s	0.30%	b	g	3.61	5.14
99	79Au176	175.98	1.3+-.3s	a	b	g	10.37	6.26
100	79Au177	176.98	1.3+-.4	100%				6.115
101	79Au178	177.98	2.6+-5	100%				5.92
102	79Au179	178.97	7.5 s	100%				5.85
103	80Hg178	177.98	0.26 s	50%		50%	6.25	6.43
104	80Hg179	178.98	1.09 s	a		g	7.88	6.29
105	80Hg180	179.98	2.9 s	a		g	5.54	6.12
106	80Hg181	180.98	3.6 s		26% b	g	7.07	
107	80Hg182	181.97	11 s		15% b	g	4.81	5.87
108	80Hg183	182.97	8.8 s	a	b	g	6.24	5.83
109	80Hg184	183.97	30.9 s		1% b	g	3.98	5.54
110	80Hg185m		21 s	a	b	g		5.37
111	81Tl184	183.98	11 s		2% b	g	9.19	6.16
112	81Tl185m		1.8 s	a			5.97	6.01
113	82Pb184	183.99	0.6 s		100%			6.63
114	82Pb185	184.99	4.1 s		100%			6.34
115	82Pb186	185.98	8 s		5% b	g	5.39	6.32
116	82Pb187m		18.3 s	a		g		6.08
117	82Pb188	187.98	24 s		22%		7.8%	4.74
118	82Pb189	188.98	51 s	a		g	6.5	5.58
119	82Pb190	189.98			0.90%	13%	86%	4.27
120	83Bi190	189.99	5.4 s		90% b	g	9.7	6.45
121	83Bi191	190.99	13 s		40% b	g	0.37	6.32
122	83Bi192	191.99	42 s		20% b	g	8.95	6.06
123	83Bi193m		3.5 s	a	b	g		6.48
124	83Bi193	192.98	64 s		60% b	g	6.58	5.91
125	83Bi194	193.98	1.8 m		0.10% b	g	7.98	
126	83Bi195m		1.5 m		6% b	g		6.11
127	83Bi195	194.98	2.8 m		0.20% b	g	5.8	5.45
128	83Bi210m		3ee6 y		100%			4.946
129	83bi211	210.99	2.14 m		99.70% 0.30%		0.584	6.279
130	83Bi212ml		25 m		93%	7%		6.3
131	83Bi212	211.99	1 hr		36%	64%	2.248	6.051
132	83Bi213	212.99	45.6 m		2%	98%	1.422	1.42
133	84Po194	193.99	0.7 s		100%			6.85
134	84Po195m		2.0 s		100%			6.7
135	84Po195	194.99	4.5 s		100%			6.61
136	84Po196	195.99	5.5 s		95% b	g		6.52
137	84Po197m		26 s		84% b	g		6.385
138	84Po197	196.99	56 s		44% b	g		6.282
139	84Po198	197.98	1.76 m		70% b	g		6.182
140	84Po199m		4.2 m		39% b	g		6.059
141	84Po199	198.98	5.2 m		12% b	g	5.67	5.952

FIGURE 1C

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	A	B	C	D	E	F	G	H
142	84Po200	199.98	11.5 m	15%	b	g	3.37	5.863
143	84Po201	200.98	15.3 m	2%	b	g	4.9	5.683
144	84Po202	201.98	44.7 m	2%	b	g	2.82	5.588
145	84Po206	205.98	8.8 d	5%		95%	1.843	5.223
146	84Po208	207.98	2.9 y	100%			5.213	4.233
147	84Po209	208.98	105 y	100%			4.976	4.624
148	84Po210	209.98	138.4 d	100%			5.407	5.304
149	84Po211m		25.5 s	100%				7.273
150	84Po211	210.99	0.52 s	100%			7.594	7.45
151	84Po212m		45.1 s	100%				11.65
152	84Po212	211.99	0.3 μ s	100%			8.953	8.784
153	84Po213	212.99	4.2 μ s	100%			8.537	8.375
154	84Po214	213	163 μ s	100%			7.833	7.686
155	84Po215	214	1.78 ms	100%			7.526	7.386
156	84Po216	216	0.15 s	100%			6.906	6.778
157	84Po217	217.01	<10 s	100%			6.662	6.539
158	84Po218	218	3.11 m	100%			6.114	6.002
159	85At196	195	0.3 s	100%				7.06
160	85At197	196.99	0.4 s	a	b	g	7.28	6.96
161	85At198m		1.5 s	25%	b	g		6.85
162	85At198	197.99	4.9 s	100%				6.75
163	85At199	198.99	7 s	92%	b	g	6.5	6.64
164	85At200m		4.3 s	20%	b	g		6.536
165	85At200	199.99	43 s	35%	b	g	8.08	6.412
166	85At210	200.99	1.48 s	71%	b	g	6.474	6.344
167	85At202	201.99	3.02 m	12%	b	g	7.21	6.135
168	85At203	202.99	7.4 m	31%	b	g	6.21	6.088
169	85At204	203.99	9.2 m	5%	b	g	6.45	5.951
170	85At205	204.99	26.2 m	10%	b	g	6.02	5.902
171	85At206	205.99	29.4 m	1%	b	g	5.881	5.703
172	85At207	206.99	1.81 h	10%	b	g	5.873	5.758
173	85At208	207.99	1.63 h	1%	b	g	5.752	5.641
174	85At209	208.99	5.41 h	4%	b	g	5.757	5.647
175	85At210	209.98	8.1 h	0.20%		99.80%	5.632	5.442
176	85At211	210.99	7.21 h	42%		58%	5.98	5.868
177	85At212m		0.12 s	100%				7.837
178	85At212	211.99	0.3 s	100%			7.828	7.681
179	85At213	212.99	0.11 s	100%			9.254	9.08
180	85At214m		0.7 μ s	100%				8.762
181	85At214	213.98	0.56 μ s	100%			8.987	8.819
182	85At215	214	100 μ s	100%			8.178	8.023
183	85At216	216	300 μ s	100%			7.947	7.8
184	85At217	217	32.3 μ s	100%			7.202	7.067
185	85At218	218	1.6 s	100%			6.883	6.695
186	85At219	219.01	54 s	100%			6.39	6.275
187	86Rn200	200	1+-2 s	98%		2%	8.08	6.909
188	86Rn201m		3.8 s	90%		10%		6.77

FIGURE 1D

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	A	B	C	D	E	F	G	H
189	86Rn201	201	7 s	80%		20%	6.86	6.721
190	86Rn202	202	9.9 s	12%		88%	6.771	6.636
191	86Rn203	202.99	45 s	66%		34%	6.629	6.498
192	86Rn204	203.99	1.24 m	68%		32%	6.546	6.417
193	86Rn205	204.99	2.83 m	23%		77%	6.39	6.262
194	86Rn206	205.99	5.67 m	68%		32%	6.384	6.258
195	86Rn207	206.99	9.3 m	23% b	9		4.62	6.126
196	86Rn208	207.98	24.4 m	60%		40%	2.88	6.14
197	86Rn209	208.99	28.5 m	17%	83%		3.93	6.039
198	86Rn210	209.99	2.4 hr	96%		4%	2.368	6.039
199	86Rn211	210.99	14.6 hr	26% b	9		2.894	5.784
200	86Rn212	211.99	24 m	100%				6.26
201	86Rn213	212.99	25 ms	100%				8.087
202	86Rn214m		7.3 ns	4%			1.626	10.63
203	86Rn214	214	0.27 μ s	100%				9.037
204	86Rn215	215	2.3 μ s	100%				8.674
205	86Rn217	217		100%				7.742
206	86Rn218	218	35 ms	100%				7.133
207	86Rn219	219.01	3.96 s	100%				6.819
208	86Rn220	220	55.6 s	100%				6.288
209	86Rn221	221.01	25 m	22%	78%		1.15	6.037
210	86Rn222	222.02	3.82 d					5.4897
211	87Fr201	201	48 ms	100%				7.388
212	87Fr202	202	0.34 s	100%				7.25
213	87Fr203	203	0.55 s	100%				7.28
214	87Fr204	204	2.1 s	100%				7.027
215	87Fr205	205	3.96 s	100%				6.914
216	87Fr206	206	16 s	100%				6.789
217	87Fr207	207	14.8 s	100%				6.766
218	87Fr208	208	59 s	77%		23%	6.96	6.636
219	87Fr209	209	50 s	89%		11%	6.778	6.646
220	87Fr210	210	3.2 m	100%				6.543
221	87Fr211	211	3.1 m	a		9	4.57	6.543
222	87Fr212	212	20 m	43%		57%	5.07	6.261
223	87Fr213	213	34.6 s	100%				6.775
224	87Fr214m		3.4 ms	100%				8.476
225	87Fr214	214	5.1 ms	100%				8.427
226	87Fr215	215	0.12 μ s	100%				9.36
227	87Fr216	216	0.7 μ s	100%				9.005
228	87Fr217	217	22 μ s	100%				8.315
229	87Fr218	218.01	0.7 μ s	100%				7.867
230	87Fr219	219	21 s	100%				7.313
231	87Fr220	220.01	27.4 s	100%				6.686
232	87Fr221	221.01	4.9 m	100%				6.341
233	87Fr222	222	14.4 m	a b			2.06	5.85
234	88Ra206	206	0.4 s	100%				7.272
235	88Ra207	207	1.3+- .2s	100%				7.133

FIGURE 1E

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	A	B	C	D	E	F	G	H
236	88Ra208	208	1.4 s	100%				7.133
237	88Ra209	209	4.6 s	100%				7.008
238	88Ra210	210	3.7 s	100%				7.02
239	88Ra211	211	13 s	100%				6.912
240	88Ra212	212	13 s	100%				6.901
241	88Ra213	213	2.7 m	80%		20%	3.88	6.622
242	88Ra214	214	2.46 s	100%				7.136
243	88Ra215	215	1.59 ms	100%				8.7
244	88Ra216	216	0.18 μ s	100%				9.349
245	88Ra217	217.01	1.6 μ s	100%				8.992
246	88Ra218	218	14 μ s	100%				8.39
247	88Ra219	219.01		100%				7.68
248	88Ra220	220.01	23.5 s	100%				7.455
249	88Ra221	221.01	28 s	100%				6.608
250	88Ra222	222.01	38 s	100%				6.556
251	88Ra223	223.01	11.43 d	100%				5.716
252	88Ra224	224.02	3.66 d	100%				5.685
253	88Ra226	226.03	1600 y	100%				4.784
254	89Ac210	210.01	0.35 s	100%				7.462
255	89Ac211	211.01	0.25 s	100%				7.48
256	89Ac212	212.01	0.93 s	100%				7.379
257	89Ac213	213.01	0.8 s	100%				7.364
258	89Ac214	214.01	8.2 s	86%		14%		7.214
259	89Ac215	215.01	0.17 s	100%				7.604
260	89Ac216m		0.33 ms	100%				9.028
261	89Ac216	216.01	0.33 ms	100%				9.07
262	89Ac217m		0.4 μ s	100%				10.54
263	89Ac217	217.01	0.11 μ s	100%				9.65
264	89Ac218	218	0.27 μ s	100%				9.205
265	89Ac219	219.01	7 μ s	100%				8.664
266	89Ac220	220.01	26.1 ms	100%				7.61
267	89Ac221	221.01	52 ms	100%				7.645
268	89Ac222m		1.1 m	>89%		1%		6.71
269	89Ac222	22.01	4.2 s	100%				7.013
270	89Ac223	223.02	2.2 m	99%		1%	0.584	6.646
271	89Ac224	224.02	2.9 hr	10%		90%	1.397	6.138
272	89Ac225	225.02	10 d	100%				5.793
273	89Ac226	226.03	1.2 d	0.01%	83%	16%	1.117	5.399
274	89Ac227	227.03	21.77 y	1.40%	96.60%		0.041	4.951
275	90Th212	212.01	30 ms	100%				7.8
276	90Th213	213.01	0.14 s	100%				7.692
277	90Th214	214.01	0.86 s	100%				7.677
278	90Th215	215.01	1.2 s	100%				7.395
279	90Th216	216.01	28 ms	100%				7.921
280	90Th217	217.01	252 μ s	100%				9.25
281	90Th218	218.01	0.11 μ s	100%				9.665
282	90Th219	219.02	1.05 μ s	100%				9.34

FIGURE 1F

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	A	B	C	D	E	F	G	H
283	90Th220	220.02	9.7 μ s	100%				8.79
284	90Th221	221.02	1.66 ms	100%				8.146
285	90Th222	222.02	2.8 ms	100%				7.982
286	90Th223	223.02	0.66 s	100%				7.287
287	90Th224	224.02	1.04 s	100%				7.17
288	90Th225	225.02	8 m	90%		10% 0.668		6.479
289	90Th226	226.02	31 m	100%				6.3338
290	90Th227	227.02	18.72 d	100%				
291	90Th228	228.03	1.91 y	100%				5.4233
292	90Th229	229.03	7.3ee3 y	100%				4.845
293	90Th230	230.03	7.5ee4 y	100%				4.6876
294	90Th232	232.04	1.4ee10 y	100%				4.01
295	91Pa216	216.02	0.2 s	100%				7.92
296	91Pa217m		1.6 ms	100%				10.16
297	91Pa217	217.02	4.9 ms	100%				8.34
298	91Pa218	218.02	0.12 ms	100%				9.54
299	91Pa222	222.02	4.3 ms	100%				8.18
300	91Pa223	223.02	6 ms	100%				8.006
301	91Pa224	224.02	0.95 s	100%				7.49
302	91Pa225	225.02	1.8 s	100%				7.245
303	91Pa226	226.03	1.8 s	74%		26% 2.834		6.863
304	91Pa227	227.03	38.3 m	85%		15% 1.02		6.465
305	91Pa228	228.03	22 h	2%		98%		6.087
306	91Pa229	229.03	1.4 d	0.20%		99.80% 0.296		5.579
307	91Pa231	231.04	3.3ee4 y	100%				5.013
308	92U226	226.03	0.5 s	100%				7.43
309	92U227	227.03	1.1 m	100%				6.87
310	92U228	228.03	9.1 m	100%				6.681
311	92U229	229.03	58 m	20%		80% 1.305		6.36
312	92U230	230.03	20.8 d	100%				5.89
313	92U232	232.04	68.9 y	100%				5.32
314	92U233	233.04	1.6ee5 y	100%				4.824
315	92U234	234.04	2.5ee5 y	100%				4.776
316	92U235	235.04	7ee8 y	100%				4.395
317	92U236	236.05	2.34ee7y	100%				4.494
318	92U238	238.05	4.46ee9y	100%				4.196
319	93Np229	229.04	4 m	100%				6.89
320	93Np230	230	4.6 m	3%		97% 3.62		6.66
321	93Np231	231.04	48.8 m	2%		98% 1.84		6.28
322	93Np235	235.04	1.08 y	0.10%		99.90% 0.123		
323	93Np237	234.05	2.14ee6y	100%				4.788
324	94Pu232	232.04	34 m	<20%		<80% 1.07		6.6
325	94Pu233	233.05		0.10%		99.90% 2.02		6.3
326	94Pu234	234.04	8.8 hr	6%		94% 0.383		6.2
327	94Pu235	235.04	25.6 m	0.01%		99.90% 1.13		5.85
328	94Pu236	236.05	2.85 y	100%				5.768
329	94Pu237	237.05	45.1 d	0.01%		99.90% 0.218		5.334

FIGURE 1G

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	A	B	C	D	E	F	G	H
330	94Pu238	238.05	87.7 y	100%				5.499
331	94Pu239	239.05	2.41 y	100%				5.156
332	94Pu240	240.05	6537 y	100%				5.168
333	94Pu241	241.06	14.4 y	a	99+%		0.021	4.897
334	94Pu242	242.06	3.76ee5y	100%				4.901
335	94Pu244	244.06	8.2ee7 y	99.90%				4.589
336	95Am237	237.05	1.22 hr	0.02%		99.98%	1.54	6.042
337	95Am239	239.05	11.9 hr	0.01%		99.99%	0.8	5.776
338	95Am240	240.06	50.9 hr	a		g	1.369	5.378
339	95Am241	241.06	432.2 y	100%				5.486
340	95Am242m		141 y	0.50%				5.207
341	95Am243	243.06	7370 y	100%				5.277
342	96Cm238	238.05	2.4 hr	<10%		<90%	0.97	6.52
343	96Cm240	240.06	27 d	100%				6.291
344	96Cm241	241.06	32.8 d	1%				5.939
345	96Cm242	242.06	462.8 d	100%				6.113
246	96Cm243	243.06	28.5 y	100%				5.788
347	96Cm244	244.06	18.11 y	100%				5.805
348	96Cm245	245.07	8500 y	100%				5.362
349	96Cm246	246.07	4780 y	100%				5.386
350	96Cm247	247.07	1.56ee7y	100%				4.896
351	96Cm248	248.07	3.4ee5 y	92%				5.078
352	97Bk243	243.06	4.5 hr	0.15%		99.80%	1.505	6.574
353	97Bk244	244.07	4.4 hr	0.01%		99.99%	2.25	6.625
354	97Bk245	245.07	4.94 d	0.10%		99.90%	0.814	5.885
355	97Bk247	247.07	1400 y	100%				5.532
356	98Cf240	240.06	1.06 m	100%				7.59
357	98Cf241	241.06	3.8 m	a		g	3.08	7.335
358	98Cf242	242.06	3.5 m	100%				7.385
359	98Cf243	243.07	10.7 m	14%		86%	2.23	7.06
360	98Cf244	244.06	19.4 m	100%				7.21
361	98Cf245	245.07	43.6 m	30%		70%	1.565	6.886
362	98Cf246	246.07	36 hr	100%				6.75
363	98Cf247	247.07	3.11 hr	0.04%		99.96%	0.67	6.301
364	98Cf248	248.07	334 d	100%				6.262
365	98Cf249	249.07	351 y	100%				5.812
366	98Cf250	250.07	13.1 y	100%				6.031
367	98Cf251	251.08	890 y	100%				5.677
368	98Cf252	252.08	2.64 d	96.90%				6.118
369	98Cf253	253.09	17.8 d	0.30%	99.70%		0.29	5.979
370	98Cf254	254.09	60.5 d	0.30%			5.93	5.834
371	99Es243	243.07	21 s	30%		70%	3.81	7.89
372	99Es244	244.07	37 s	4%		96%	4.5	7.57
373	99Es245	245.07	1.33 m	40%		60%	3	7.73
374	99Es246	246.07	7.7 m	10%		90%	3.84	7.35
375	99Es247	247.07	4.7 m	7%		93%	2.4	7.32
376	99Es248	248.08	27 m	0.30%		99.70%	3.03	6.87

FIGURE 1H

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	A	B	C	D	E	F	G	H
377	99Es249	249.08	1.7 hr	0.60%		99.40%	1.395	6.77
378	99Es251	251.08	1.38 d	0.50%		99.50%	0.379	6.492
379	99Es252	252.08	1.29 d	76%		24%	1.12	6.632
380	99Es253	253.08	20.47 d	100%				6.633
381	99Es254m		1.64 d	0.30%	99.60%		1.16	6.382
382	99Es254	254.09	275 d	100%				6.427
383	99Es255	255.1	39.8 d	8%	92%		0.3	6.3
384	100Fm243	243.07	0.18 s	100%				8.546
385	100Fm245	245.08	4 s	100%				8.15
386	100Fm246	246.08	1.1 s	92%			8.373	8.24
387	100Fm247m		9.2 s	100%				8.18
388	100Fm247	247.08	35 s	100%				7.87
389	100Fm248	248.08	36 s	99.90%				7.87
390	100Fm249	249.08	2.6 m	a		g		7.53
391	100Fm250	250.08	30 m	100%				7.43
392	100Fm251	251.08	5.3 hr	2%		98%	1.49	6.832
393	100Fm252	252.08	25.4 hr	100%				7.04
394	100Fm253	253.09		12%		88%	0.334	6.943
395	100Fm254	254.09	3.24 hr	99+%				7.189
396	100Fm255	255.09	20.1 hr	100%				7.023
397	100Fm256	256.09	2.63 hr	18%				6.92
398	100Fm257	257.08	100.5 d	99.80%				
399	101Md248	248.08	7 s	20%		80%	5.21	8.32
400	101Md249	249.09	24.4 s	>20%		<80%	3.76	8.03
401	101Md250	250.08	52 s	6%		94%	4.54	7.75
402	101Md251	251.08	4 m	<6%		>94%	3.02	7.55
403	101Md252	252.09	2.3 m	50%		50%	3.73	7.73
404	101Md255	255.09	27 m	8%		92%	1.06	7.326
405	101Md256	256.09	76 m	10%		90%	2.041	7.22
406	101Md257	257.1	5.2 hr	10%		90%	0.45	7.068
407	101Md258	258.1	56 d	100%				6.716
408	102No251	251.09	0.8 s	100%				8.6
409	102No252	252.09	2.3 s	73%			8.551	8.415
410	102No253	253.09	1.7 m	100%				8.01
411	102No254	254.09	55 s	100%				8.1
412	102No255	255.09	3.1 m	62%		38%	8.445	8.121
413	102No256	256.09	3.2 s	100%				8.43
414	102No257	257.1	25 s	100%				8.322
415	102No259	259.1	58 m	78%		22%	7.794	7.488
416	103Lr253	253.1	1.4 s	100%				8.721
417	103Lr254	254.1	20 s	100%				8.455
418	103Lr255	255.1	22 s	100%				8.37
419	103Lr256	256.1	28 s	99.70%				8.43
420	103Lr257	257.1	0.65 s	100%				8.861
421	103Lr258	258.1	4.3 s	100%				8.589
422	103Lr259	259.1	5.4 s	100%				8.46
423	103Lr260	260.11	3 m	100%				8.04

FIGURE 11

	A	B	C	D	E	F	G	H
424	104Rf257	257.1	4.8 s	100%				8.663
425	104Rf259	259.11	3.1 s	100%				8.77
426	104Rf261	261.11	65 s	100%				8.29
427	105Ha257	257.11	1 s	100%				8.96
428	105Ha258	258.11	4 s	100%				9.02
429	105Ha260	260.11	1.5 s	a				9.05
430	105Ha261	261.11	1.8 s	a				8.93
431	105Ha262	262.11	34 s	a				8.45
432	106--261		1 ms	100%				
433	106--262		115 ms	100%				9.7

FIGURE 1J

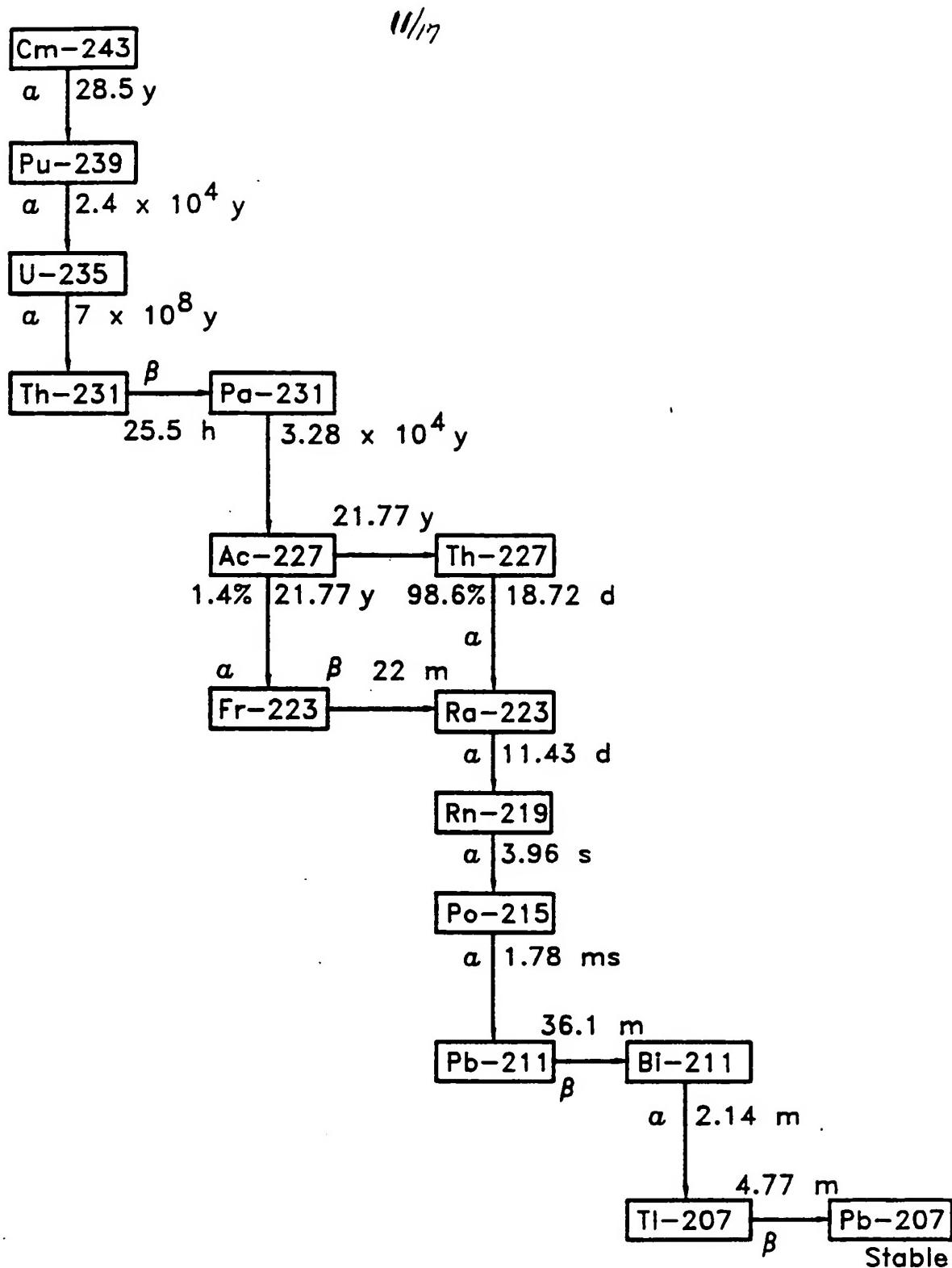


FIGURE 2

CONTINUED SHEET

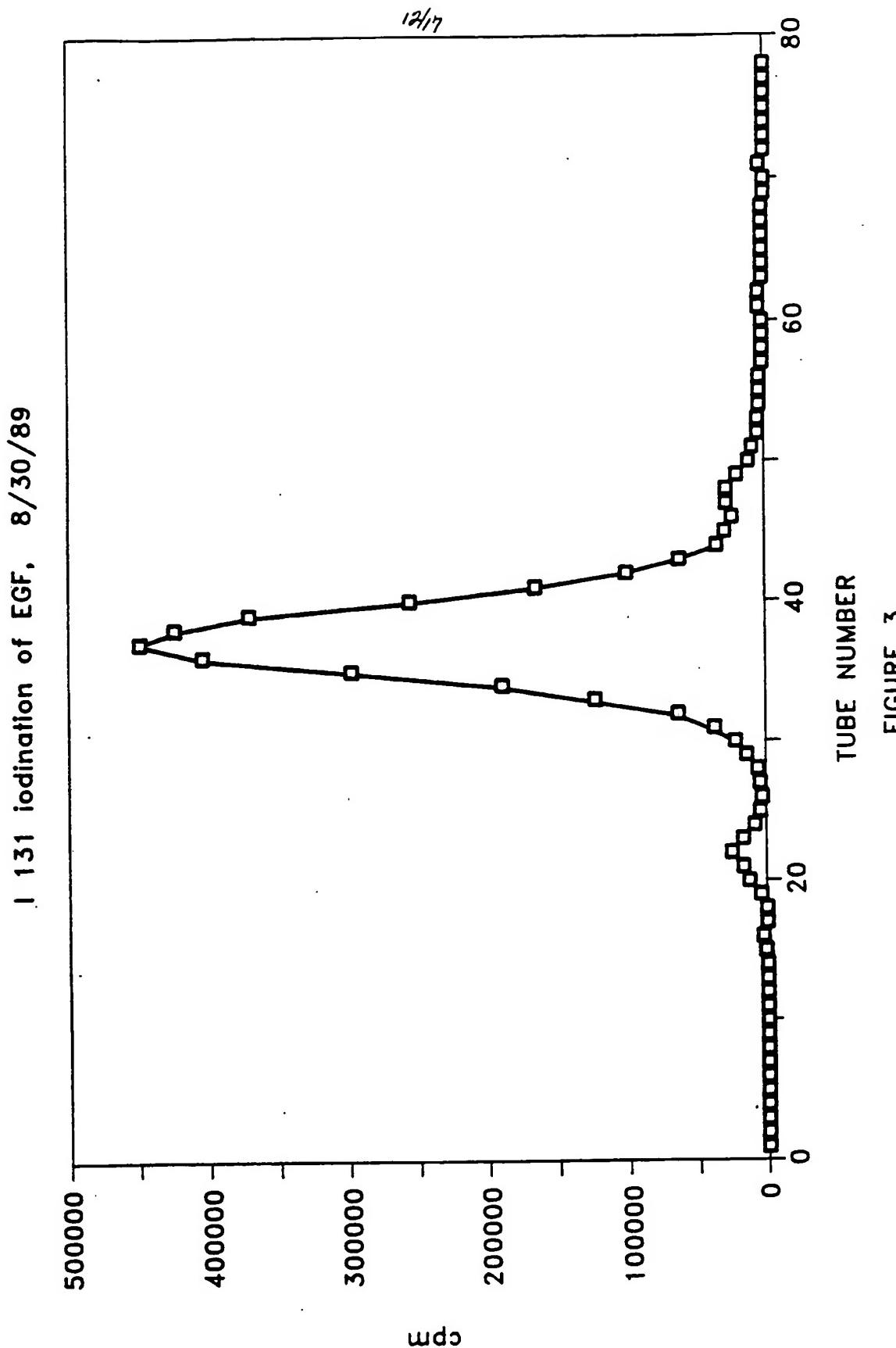


FIGURE 3

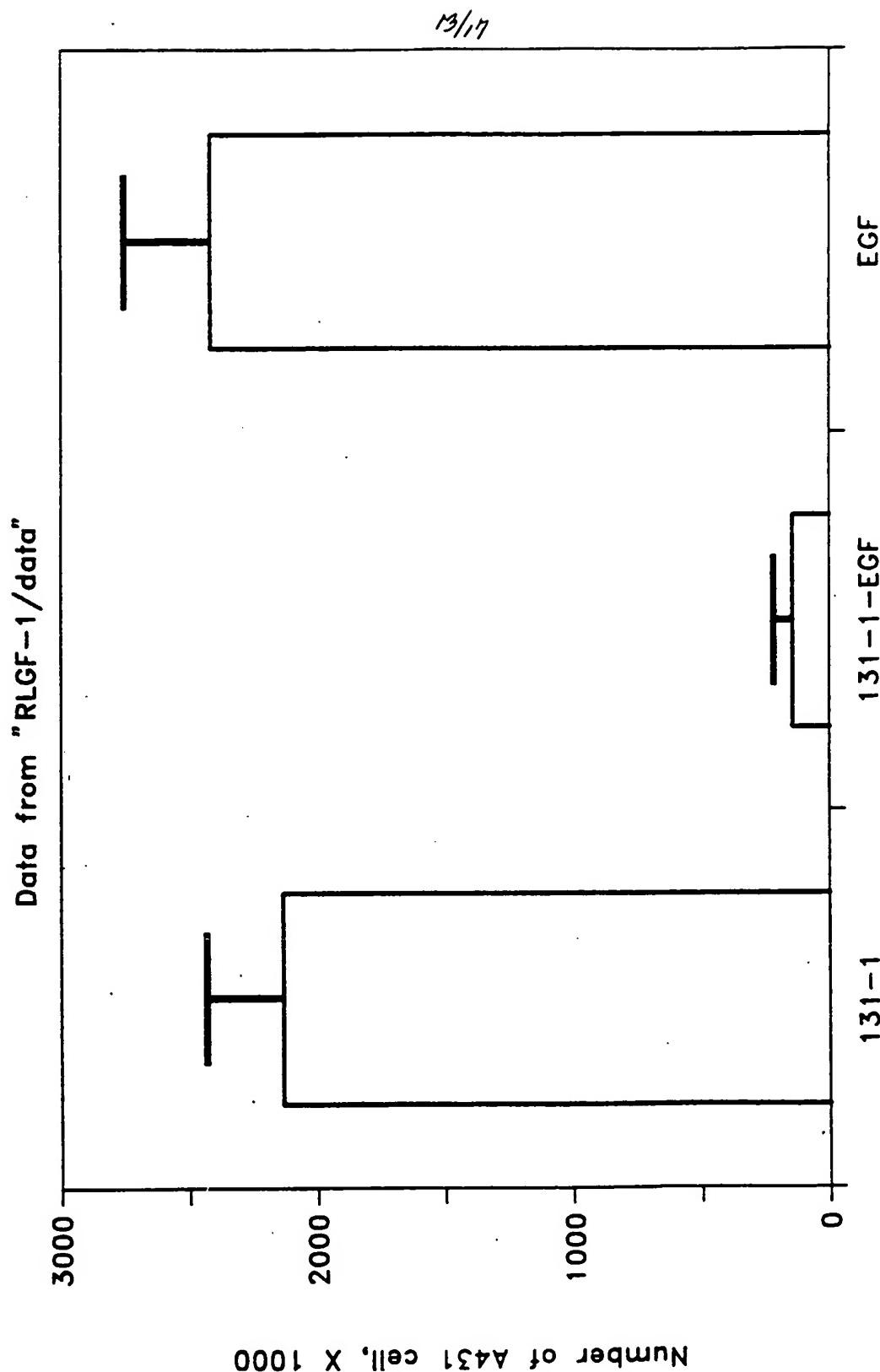
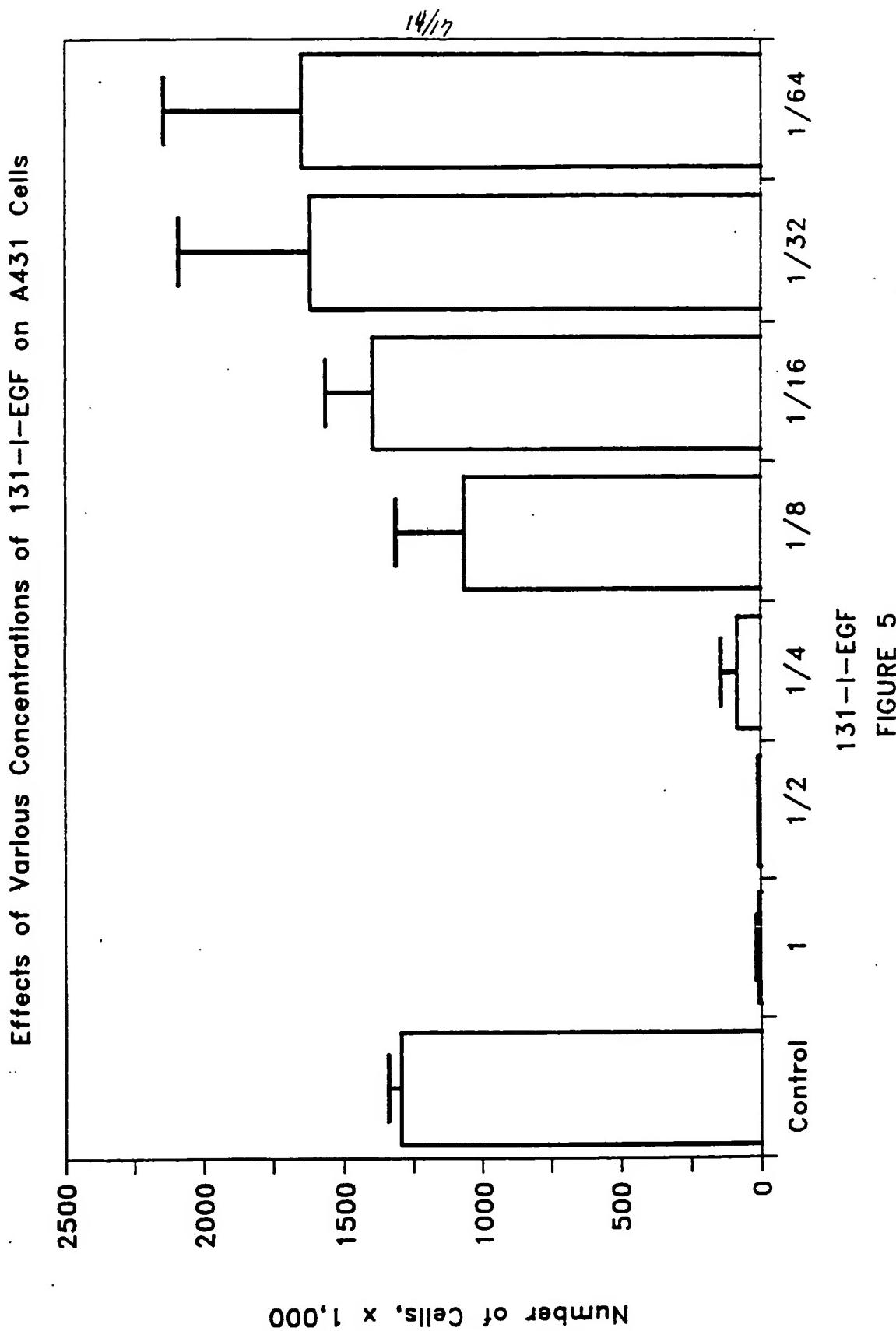
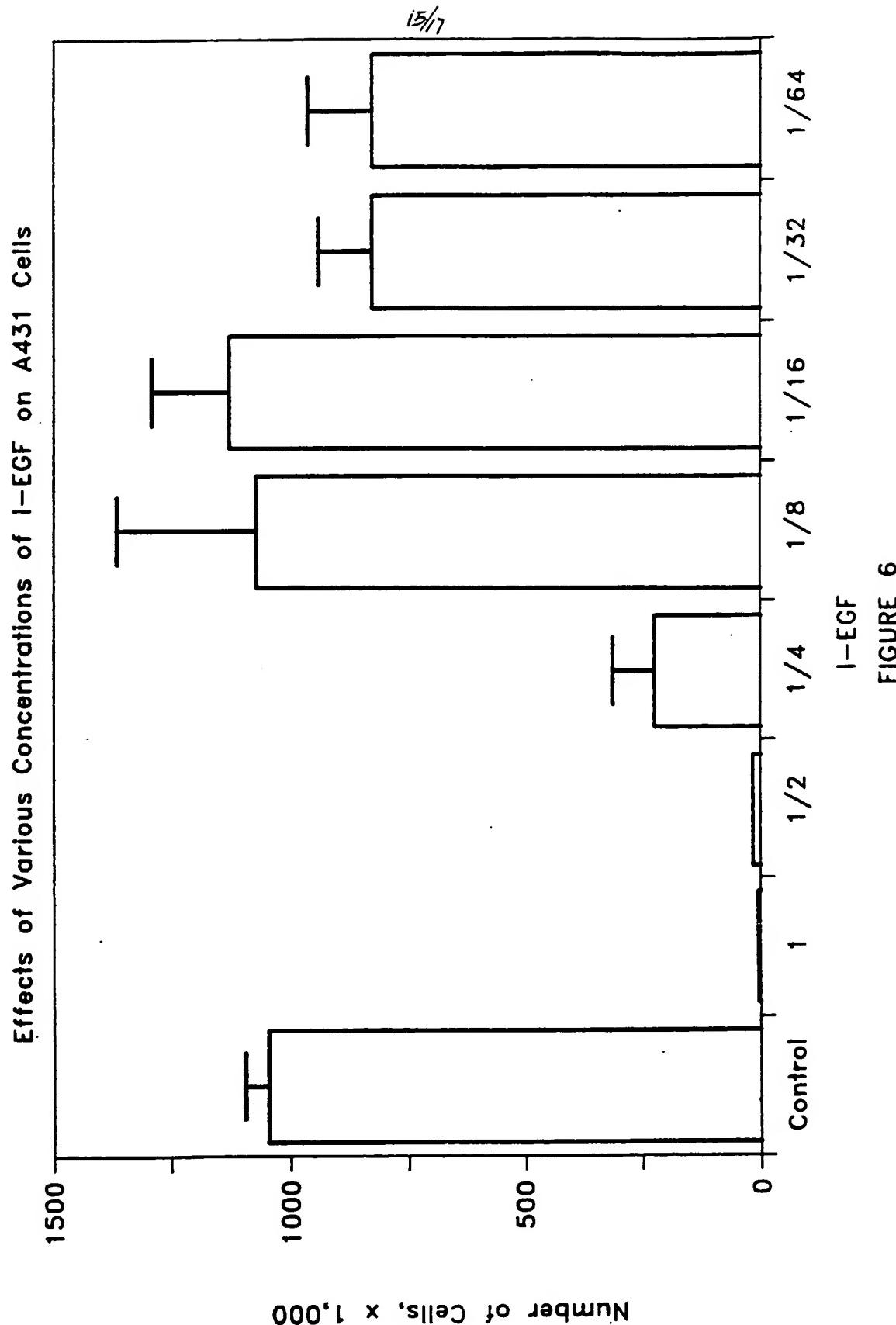
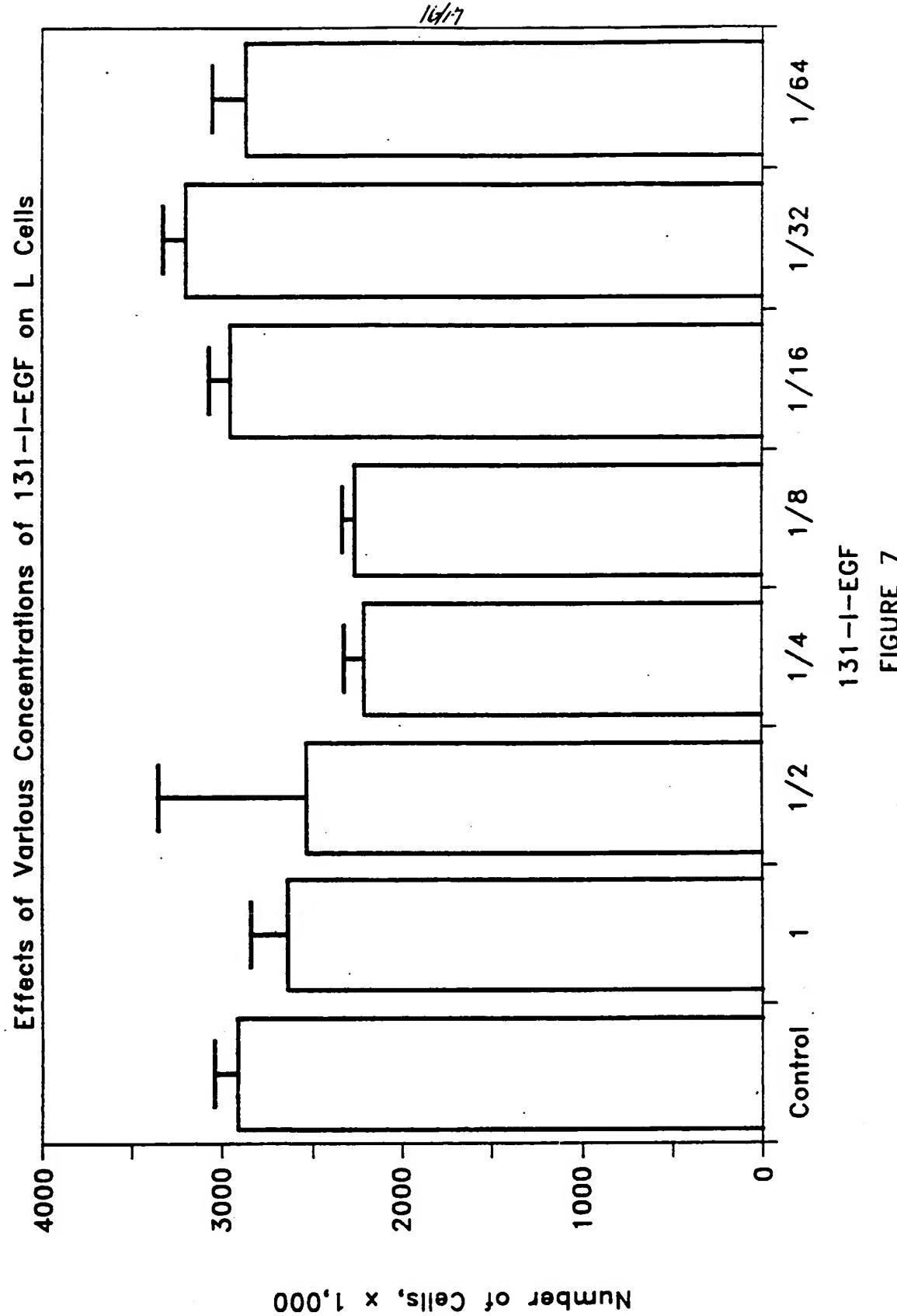


FIGURE 4



SUBSTITUTE SHEET





SUBSTITUTE SHEET

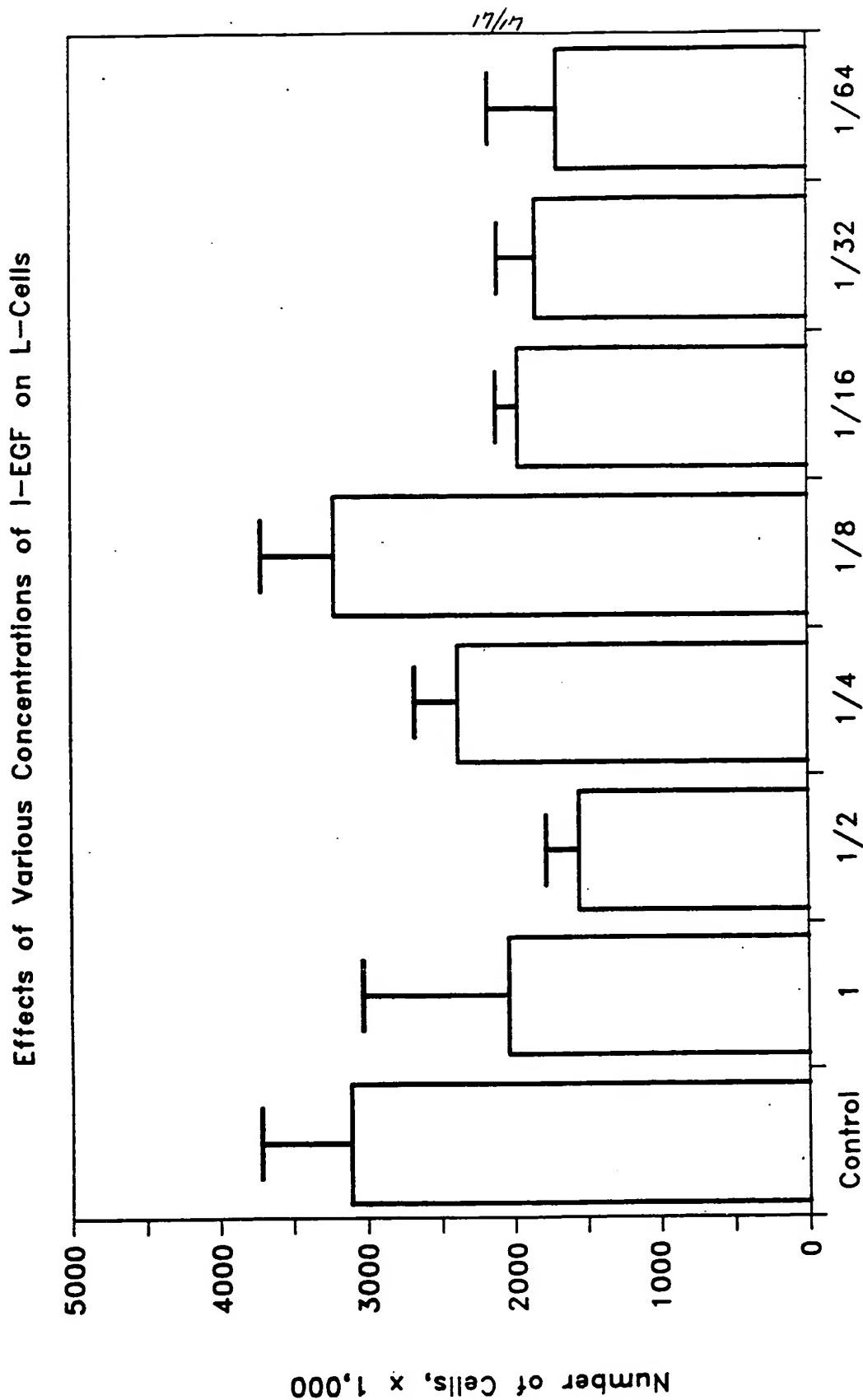


FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/09874

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 49/00, A61K 49/00, A61K 43/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US, A, 5135736 (D. C. ANDERSON ET AL.), 4 August 1992 (04.08.92), see esp. col. 3, 1. 28-49 and col. 5, 1. 34-45 --	1-16,31-34
X	US, A, 5037630 (A. R. FRITZBERG ET AL.), 6 August 1991 (06.08.91), see esp. col. 7, 1. 35 --	1-16,31-34
X	WO, A1, 9101144 (SANDOZ-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H.), 7 February 1991 (07.02.91), see esp. p. 3, 6 and p. 26 --	1-16,31-34

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

19 March 1993

Date of mailing of the international search report

13.04.93

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ANNA SJÖLUND

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/09874

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4988496 (A. SRINIVASAN ET AL.), 29 January 1991 (29.01.91), see esp. col. 7, 1. 60 - col. 8, 1. 21 --	1-16,31-34
X	US, A, 5059541 (A. R. FRITZBERG ET AL.), 22 October 1991 (22.10.91), see esp. col. 2, 1. 65 - col. 3, 1. 64 ----	1-16,31-34

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 17-30
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

SA. 7890

INTERNATIONAL SEARCH REPORT

Information on patent family members

26/02/93

International application No.

PCT/US 92/09874

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 5135736	04/08/92	DE-U-	6890401	04/02/93
		EP-A,B-	0359347	21/03/90
		JP-A-	2124833	14/05/90
		US-A-	5169933	08/12/92
US-A- 5037630	06/08/91	US-A-	4897255	30/01/90
		US-A-	5120526	09/06/92
		DE-A-	3680924	26/09/91
		EP-A,B-	0188256	23/07/86
		JP-A-	61225163	06/10/86
		US-A-	5175343	29/12/92
WO-A1- 9101144	07/02/91	AU-A-	6070990	22/02/91
		EP-A-	0436005	10/07/91
		JP-T-	4500823	13/02/92
US-A- 4988496	29/01/91	EP-A-	0344724	06/12/89
		US-A-	5075099	24/12/91
US-A- 5059541	22/10/91	EP-A-	0339684	02/11/89